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REPRODUCTIVE TACTICS OF APHIDOPHAGOUS LADY BEETLES:
COMPARISON OF A NATIVE SPECIES AND AN INVASIVE
SPECIES THAT IS DISPLACING IT

by

Yukie Kajita

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

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2008

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ABSTRACT

Reproductive Tactics of Aphidophagous Lady Beetles: Comparison of a Native Species
and an Invasive Species That Is Displacing It

by

Yukie Kajita, Doctor of Philosophy

Utah State University, 2008

Major Professor: Dr. Edward W. Evans
Department: Ecology

Coccinella septempunctata L. (Coleoptera: Coccinellidae) has been introduced to North America in recent decades, raising concerns of adverse impacts on native lady beetles, including the congeneric *C. transversoguttata richardsoni* (Brown). The central focus of my dissertation is to understand the importance of reproduction, in particular, in promoting invasion of *C. septempunctata* and its replacement of native lady beetles in alfalfa fields of western North America.

Studies were conducted to compare reproductive tactics of the invasive *C. septempunctata* and the native *C. transversoguttata*, by addressing: 1) maximum rate of reproduction of overwintered lady beetles, 2) population dynamics of the invasive and native lady beetles and their aphid prey, and seasonal reproductive patterns, over a 3-year period (2004–2006) in alfalfa fields, 3) reproductive tactics and plasticity in response to various prey availabilities, and 4) ovarian dynamics and observation of oosorption in response to prey removal.

The invasive *C. septempunctata* gained a reproductive advantage over native, North American lady beetles from its larger body size when feeding on abundant prey. The invasive species gained additional advantage by its allocation of prey to larger numbers of relatively small eggs. In alfalfa fields, females of *C. septempunctata* reproduced more readily and laid more eggs than females of *C. transversoguttata* even at low prey density. *C. septempunctata* females collected from the field were also more successful in approaching their maximum body weights and reproduction, as observed under ideal conditions, than were females of native *C. transversoguttata*. In the laboratory, *C. septempunctata* females produced larger numbers of relatively small eggs, and they maintained their body weights even as they were producing eggs at low rates when aphids were provided in limited numbers. More immediate adjustment of reproductive effort with prey removal, and higher recovery of reproductive rate when prey again became available, were observed in *C. septempunctata*, compared with *C. transversoguttata*. These reproductive abilities of *C. septempunctata* may contribute to its invasion success and dominance in alfalfa fields. Further studies are needed to determine why females of *C. septempunctata* are in better physiological condition than are females of *C. transversoguttata* in spring alfalfa fields.

(170 pages)

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Yukie Kajita

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CHAPTER 1

INTRODUCTION

In this dissertation, I address important issues in invasion ecology by examining the reproductive behavior of an invasive lady beetle (Coleoptera, Coccinellidae) that was introduced to North America for biological control. I examine potential mechanisms that might account for the invasion success of this species by focusing especially on life history traits that may promote reproduction by this species. Throughout, I seek perspective by comparing the performance of this species with the performance of a congeneric, native North American species. Here I introduce major themes of this dissertation, and I outline my research questions and objectives.

Invasion Ecology and Biological Control

Global biodiversity and the functioning of ecological communities are threatened by invasive species brought to new geographic regions of the world by human transport and activities and by the consequent global homogenization of the biosphere (e.g., Lodge 1993; Mack et al. 2000). With adoption of the biodiversity treaty by the United Nations Conference on Environment and Development (UNCED) in 1992, the need has been recognized worldwide to conserve biological diversity and to use biological resources in a sustainable manner. In the United States, the National Invasive Species Council (NISC) was established by the federal government in 1999 to manage invasive species from both economic and ecological perspectives, and for human health. The term “invasive species” has been defined as “a non-native species whose introduction does or is likely to cause economic or environmental harm or harm to human, animal, or plant health” by

Federal register (Executive order 13112, 1999). “Environmental harm” includes biologically significant decreases in native species populations, alterations to plant and animal communities, or to ecological processes that native species and other desirable plants and animals and humans depend on for survival (National Invasive Species Council 2006).

Biological control is a method of agricultural pest management that involves promotion of natural enemies, such as predators, parasitoids, herbivores and pathogens to reduce pest numbers. In classical biological control, natural enemies are introduced from the geographic region where an exotic pest originates. For example, invasive prickly pear cactus (*Opuntia inermis* and *O. stricta*) from South America was controlled successfully in Australia by introduced cactus moth (*Cactoblastis cactorum*) from Argentina (Osmond and Monro 1981). Similarly, introduced alligator weed (*Alternanthera phytolaxeroides*) in Florida and Georgia was controlled by introduced flea beetles (Center et al. 1997; also see Mack et al. 2000). Because introduced natural enemies can usually sustain themselves without human intervention, costs and efforts for continuing pest management are low. Therefore, biological control is often a preferred alternative to chemical control of agricultural insect pests.

One recurring theme concerning introduced exotic natural enemies in classical biological control is adverse non-target effects of exotic natural enemy on native species in local communities (e.g., Howarth 1991; Simberloff and Stiling 1996; Thomas and Willis 1998; Louda et al. 2003). Introduced biological control agents have the potential to become “invasive species” rather than simply playing their intended role as beneficial organisms in agricultural pest management. However, it is often not well understood how

local communities and ecosystems are disturbed by such invasive species.

Predatory Lady Beetles as Biological Control Agents

Since the 1888 introduction of the Australian vedalia lady beetle (*Rodolia cardinalis*) from New Zealand for the control of cottony-cushion scale insect (*Icerya purchasi*) in California, predatory lady beetles (or ladybirds; Coleoptera: Coccinellidae) have been of much interest as biological control agents in pest management (e.g., Dixon 2000). A number of species of predatory lady beetles have been introduced to North America for management of agricultural pests (e.g., aphids) in recent decades, raising concerns of adverse impacts on native species, especially native lady beetles (e.g., Evans 1991, Louda et al. 2003). A prominent species among these introduced lady beetles is *Coccinella septempunctata* L. (Angalet et al. 1979). Introduction of *C. septempunctata* to North America began in 1956 with the release of individuals (from India) in California, followed by additional introductions in the mid-western United States between 1958 and 1973 (Angalet et al. 1979; also see Louda et al. 2003). With multiple introductions, no genetic loss or bottle neck events have been observed in North American populations of *C. septempunctata*, as originated from Palearctic populations (Krafsur et al. 1992, 2005). There are several reports that the densities of native predatory lady beetles, and in particular those of *C. transversoguttata richardsoni* (Brown), have declined as these species have been displaced by introduced *C. septempunctata* in North America (e.g., Elliott et al. 1996; Turnock et al. 2003; Alyokhin and Sewell 2004). At the present time, the introduction of additional non-native coccinellids as part of federal biological control programs has been stopped because of unexpected, adverse consequences resulting from

the introductions of *C. septempunctata* and other invasive lady beetles (Louda et al. 2003). Attention has been focused instead on determining the extent of non-target effects of previously introduced, “invasive” lady beetle species such as *C. septempunctata* on local biotic communities in North America.

In northern Utah, *C. septempunctata* first appeared in 1991 and now it is predominant among predatory lady beetles in alfalfa (Evans 2000, 2004). The origin of *C. septempunctata* in northern Utah is unknown. Releases of *C. septempunctata* in Utah were made in 1978 from source populations in Meadowlands, East Rutherford, NJ (Bergen Co.) (Angalet et al. 1979), but these releases are thought not to have been successful (D. W. Davis, personal communication by E. W. Evans). Since the arrival of *C. septempunctata* in alfalfa fields of northern Utah, the native, congeneric species *C. transversoguttata* has become much less abundant in this habitat (Evans 2000, 2004). Studies of mechanisms responsible for success of this invasive lady beetles in northern Utah and other parts of North America have mainly focused on intraguild predation, but the importance of intraguild predation in field settings is still unclear (e.g., Obrycki et al. 1998; Kajita et al. 2000, 2006; Snyder et al. 2004; Yasuda et al. 2004).

Mechanisms of Invasion

There are several reports that describe the mechanisms of successful establishment of invasive species, and also the mechanisms of decline of native species as caused by invasive species. For example, predation and competition with native species for resources are the most often described mechanisms or interactions between invasive and native species (e.g., Mack et al. 2000). The success and rate of spread of an invasive

species sometimes largely depends on its excellent dispersal ability or on rapid adaptation to new conditions (Smith et al. 1999). It may also depend on the physiological capacity to tolerate and thrive under a broad spectrum of environmental conditions (e.g., Baker 1974; Sakai et al. 2001). In addition, native species may decline relative to invasive species because they are more susceptible to parasites or pathogens (Sakai et al. 2001) or because they are vulnerable hybridization with invasive species (Rozhnov 1993).

Insect Reproduction and Life History Traits

How successfully invasive species are able to reproduce as well as survive in a new environment is significant for their establishment. Life history traits associated with successful invasive species have been of much interest in recent decades, especially as these may allow prediction of which species have the greatest potential to become invasive. The relationship between rates of successful invasion and reproductive traits has been examined in insects, especially introduced natural enemies of pest species (Crawley 1986), and in plants (Rejmanek and Richardson 1996).

Insects have specialized ovaries and vitellogenic mechanisms to produce eggs, which have enabled them to diversify successfully throughout the world (Papaj 2000). In general, the female reproductive system includes a pair of ovaries with a pair of lateral oviducts. Each ovary has ovarioles, or egg-tubes, in which oocytes develop (Chapman 1998). There are two major groups of reproductive systems in insects; 1) “synovigenic” (in which females mature eggs throughout their lives), and 2) “pro-ovigenic” (wherein females emerge with the entire potential lifetime complement of eggs mature) (e.g., Papaj 2000). Predatory lady beetles are synovigenic species; therefore, prey availability during

adult life is a key for their successful reproduction (e.g., Dixon 2000). Aphidophagous lady beetles require aphids as food to maintain themselves as adults and to reproduce (e.g., Evans and Dixon 1986; Richards and Evans 1998). Aphid populations are ephemeral in natural settings; therefore, females need to forage widely and adapt to such heterogeneous environments for their survival and for their progeny's survival.

Research Objectives and Questions

The central focus of this research is to compare reproductive habits and tactics of the invasive *Coccinella septempunctata* with those of *C. transversoguttata*, a closely related native species that has been displaced in North America (e.g., Elliott et al. 1996; Turnock et al. 2003; Alyokhin and Sewell 2004). I conducted studies in alfalfa fields near Logan, Utah, where such species displacement has previously been reported (Evans 2000, 2004). Alfalfa was first introduced into western North America in the mid-1800s (Russell 2001), and fields of this crop supports over a dozen species of lady beetles in early spring (Parks 1913). I addressed two general questions in the research presented here: 1) how are the reproductive tactics of lady beetles adapted to exploiting their prey?, and 2) do the two species differ in their reproductive tactics in ways that may contribute to species displacement?

In Chapter 2, overwintered adults of *C. septempunctata* and *C. transversoguttata*, as well as of three other native lady beetles (*Hippodamia convergens*, *H. quinquesignata*, and *H. sinuata*), are compared for their reproductive rates when provided with excess numbers of aphids. I discuss relationships between body size, fecundity and invasion success based on results. In Chapter 3, the results of field censuses of alfalfa in northern

Utah are presented to compare the population dynamics of lady beetles (invasive and native species) and their aphid prey, and the seasonal reproductive patterns of lady beetles over a 3-year period (2004–2006). I consider the possibility that alfalfa fields may serve as highly suitable, core habitat for the invasive species that can promote regionally high numbers of *C. septempunctata*. In Chapter 4, reproductive responses of *C. septempunctata* and *C. transversoguttata* are compared among females provided with different levels of prey availability. Finally, in Chapter 5, ovarian and reproductive dynamics (including oosorption) in response to removal and subsequent addition of prey are examined for *C. septempunctata* and *C. transversoguttata*. These studies enable me to conclude by discussing the importance of reproduction in particular in promoting the successful invasion of *C. septempunctata* and its displacement of lady beetles in alfalfa fields of western North America.

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CHAPTER 2

**REPRODUCTIVE COMPARISON BETWEEN AN INVASIVE SPECIES AND
NATIVE SPECIES: RELATIONSHIPS OF BODY SIZE,
FECUNDITY, AND INVASION SUCCESS**

Abstract High fecundity may promote the ability of species to invade. Within groups of similar organisms, fecundity often is positively associated with body size; invasive species tend to be large; and invasion success is often greatest in highly productive environments in which the benefits of large body size in enhancing fecundity are most likely to be realized. However, the relationships of body size, fecundity and invasion success are not well understood. These relationships were examined here by comparing reproductive potentials of a large invasive species and a set of smaller, native species that have been displaced from a habitat in which the invader has attained high density. Overwintered adults of the invasive lady beetle, *Coccinella septempunctata* L., and the native lady beetles, *C. transversoguttata richardsoni* (Brown), *Hippodamia convergens* (Guerin), *H. quinquesignata* (Kirby), and *H. sinuata crotchi* (Casey) were collected in early spring from alfalfa fields in northern Utah and were compared for reproductive parameters when provided with excess numbers of their principal prey in these fields, pea aphids. Reproductive traits measured for females of the five species were rate of egg production, egg volume, egg viability, number of ovarioles, and total egg volume laid per day. Among species, the number and total volume of eggs (number x mean egg volume) produced per day increased with increasing female size and were greatest for *C. septempunctata*, the largest species. These rates of reproduction also increased with increasing female size within species. In addition, mean volume of individual eggs

within species increased with increasing female size within species, but to a much lesser degree (common slope for all species combined, 0.15, for data standardized following Honek [1993], versus a similar common slope: 0.89, for the increase in total volume of eggs laid with an increase in female size). Although there was considerable overlap in body size, females of *C. septempunctata* on average were larger than females of the largest native species, *C. transversoguttata*. Females of these two species that were the same size laid similar total volumes of eggs per day, but females of the invasive species had more ovarioles and laid larger numbers of individually smaller eggs per day. In sum, it appears that the invasive lady beetle, *C. septempunctata*, gains a reproductive advantage over native, North American lady beetles, from its larger body size when feeding on abundant prey in a habitat in which it predominates. The invasive species may gain additional advantage in this habitat over the similarly sized, congeneric, native species *C. transversoguttata* through its allocation of resources to larger numbers of relatively smaller eggs. Thus, high fecundity may be a factor promoting the dominance of the introduced species over native lady beetles in alfalfa fields of western North America.

Introduction

An increasingly large number of species has become invasive throughout the world, and these species reach high abundance in areas to which they have been introduced (e.g., Elton 1958; Mack et al. 2000). Ecologists have taken great interest in life history traits that may allow species to be invasive, because of the potential predictive power of such traits. One recurring theme is that invasive species may have

high fecundity (e.g., Crawley 1986; Rejmanek and Richardson 1996). Within a group of similar organisms, larger body size often confers greater fecundity (Stewart et al. 1991b; Honek 1993; Jablonski 1996). Brown and Sibly (2006) point out that large body size may be favored by natural selection in environments where food is abundant, even though larger organisms have lower rates of production (including reproduction) as measured on a mass-specific basis. Habitats with abundant food often appear especially subject to invasion by introduced species (e.g., Tilman 1999). Such habitats may favor large body size, with its reproductive advantage, and this in turn may account in part for why invasive species often tend to be large (Roy et al. 2001, 2002; Brown and Sibly 2006). Invasive species may gain further reproductive advantage through the trade-off between number and size of eggs (Smith and Fretwell 1974; Montague et al. 1981; Berrigan 1991; Stewart et al. 1991b) by allocating a given amount of reproductive resources to more but individually smaller eggs (versus fewer, larger eggs).

Here, the reproductive capacities of an invader and its native competitors are compared in an environment in which food can often be very abundant, and in which the relatively large invader has replaced the smaller native species as the most abundant species present. The hypothesis is tested that the introduced and now dominant lady beetle *Coccinella septempunctata* L. (Coccinellidae) has the potential for higher fecundity than four native species of lady beetles with which it co-occurs in alfalfa fields of western North America (Evans 2000, 2004). Thus, the reproductive advantage that the introduced species may gain by its larger size when feeding on abundant prey is assessed. Further, it is tested whether the introduced species gains additional advantage through its allocation of resources to increased egg number at the expense of reduced

egg size. The test for trade-offs in egg number versus egg size includes consideration that reproductive characteristics may vary with body size within as well as among species (e.g., Honek 1993; Dixon 2000).

The study system

Since its introduction in the mid nineteenth century (Russelle 2001), alfalfa has been grown widely throughout the intermountain west of North America. Introduced pest insects, including the pea aphid (*Acyrtosiphon pisum*, Hemiptera: Aphididae), often reach high numbers in alfalfa fields, and support abundant predators (e.g., Evans and Youssef 1992). Common predators include diverse species of lady beetles (Coleoptera: Coccinellidae) that feed primarily on aphids (e.g., Evans 1991). The lady beetle fauna now includes *C. septempunctata*, a recently established species in North America (Schaefer et al. 1987). In agro-ecosystems throughout North America, densities of native lady beetles, especially *C. transversoguttata richardsoni* (Brown), appear to have declined as they have been displaced by the invasive *C. septempunctata* (e.g., Elliott et al. 1996; Turnock et al. 2003; Alyokhin and Sewell 2004). In my research sites, alfalfa fields of northern Utah, *C. septempunctata* first appeared in 1991 and it is now the predominant species among lady beetles (Evans 2000, 2004). Intraguild predation has been hypothesized as a major mechanism accounting for such dominance of *C. septempunctata*, but the importance of intraguild predation in field settings is still unclear (e.g., Obrycki et al. 1998; Kajita et al. 2000, 2006; Snyder et al. 2004; Yasuda et al. 2004). Another hypothesis is that *C. septempunctata* is especially well suited in its reproductive characteristics to thrive in alfalfa (a habitat that it also

inhabits in large numbers in its native range in Europe; Hodek and Honek 1996). To test this general hypothesis, the reproductive behavior of *C. septempunctata* was compared with four species of native North American lady beetles that also inhabit alfalfa fields of northern Utah (Evans 1991, 2004): the similarly sized and congeneric *C. transversoguttata richardsoni* (Brown), and the three smaller species *Hippodamia convergens* (Guerin), *H. quinquesignata* (Kirby), and *H. sinuata crotchi* (Casey). Reproductive behaviors of the five species were compared by providing them with an abundance of pea aphids, the prey that they consume most frequently in alfalfa.

Materials and Methods

Experimental comparison of reproductive capacities

Adults of the five lady beetle species were collected from alfalfa fields near Logan, Utah, in early spring 2007 (late April to early May). These adults had recently arrived in alfalfa fields from overwintering sites, and with few exceptions (see Results below), were in the early stages of becoming reproductively active. In the lab, they were immediately mated and offered pea aphids *ad libitum* every day. 50 pairs of *Coccinella septempunctata*, *C. transversoguttata*, and *Hippodamia convergens*, 30 pairs of *H. quinquesignata*, and 12 pairs of *H. sinuata* were used for the experiment. Each pair was placed in a Petri dish (5.7cm in diameter and 1.5cm in height) in an incubator at 22°C, 16L:8D, for 20 days. On each day, each pair of lady beetles was transferred to a new Petri dish provisioned with an excess amount of pea aphids [reared on fava bean, *Vicia faba* L. (Fabaceae), in the greenhouse]. One drop of water was also added on the lid to maintain humidity inside of the Petri dish. A strip of folded filter paper was put into

each Petri dish, to create more oviposition sites for a female, to minimize egg cannibalism, and to reduce male disturbance of ovipositing females. Females that died during experiments were not replaced, and associated data were removed for analyses. Dead males were replaced, however, so that females remained paired with a live male throughout the experiment. The final sample sizes (excluding the number of dead females) were 44 pairs for *C. septempunctata*, 42 for *C. transversoguttata*, 44 for *H. convergens*, 20 for *H. quinquesignata*, and 12 for *H. sinuata*.

Wet weights of females were measured on the day of collection, and on Day 5, 10, 15, and 20 of the experiment. Reproduction by females was quantified daily. The number of eggs was counted after Petri dishes were changed. Egg cannibalism occurred infrequently. When egg cannibalism was observed, the residues of eggs were counted and added to daily egg production data. The length and width of 5 randomly selected eggs from one egg batch produced by each female were measured, using a SZX Olympus stereomicroscope at 30 x magnification at every fifth day during the experimental period. Because some females did not lay eggs on any given day, egg sizes could not be determined for all females on all days. Egg volume (mm^3) was calculated as done by Phoofolo et al. (1995) and Richards and Evans (1998) using the formula for a prolate spheroid, $(4/3)\pi ab^2$, where a is equal to half the egg length and b is equal to half the egg width. Mean egg volume from each sample of 5 eggs was calculated. Egg viability was measured at every fifth day by checking for the emergence of hatchlings at every 4 to 6 h. To prevent sibling egg cannibalism, hatchlings were removed immediately. Females were frozen at the end of the experiment. Subsequently, the length of an elytron, the pronotum and the abdomen of each female were measured.

Females were dissected to determine the number of ovarioles in each ovary. Counts for right and left ovaries were combined to obtain the total number of ovarioles for each female.

Data analyses

Body weight of each female was estimated as her mean wet weight from measurements on Day 10, 15, and 20. By Day 10 (i.e., after provision of excess prey for ten days), mean wet weight of the experimental cohort of females had stabilized for each of the five species (Fig. 2-1). Taking the mean of three measurements from Day 10, 15 and 20 for each female served to minimize the effects of variation among females in weight at the time of measurement associated with how recently they had oviposited or fed (e.g., Dixon and Guo 1993). Body weight was used as a proxy variable for body size of an individual (see Honek 1993). It was strongly positively correlated with each of the three linear measurements of body size (i.e., lengths of elytra, pronotum, and abdomen) for each of the five species [for all three linear measurements, $r=0.4\sim0.5$, $P<0.01$ for *C. septempunctata*; $r=0.6\sim0.9$, $P<0.01$ for *C. transversoguttata*; $r=0.7\sim0.9$, $P<0.01$ for *H. convergens*; $r=0.8\sim0.9$, $P<0.01$ for *H. quinquesignata*, and $r=0.5\sim0.8$, $P<0.05$ for *H. sinuata*, except for pronotum ($P=0.09$)].

Following Stewart et al. (1991b), the mean number of eggs laid by an individual female per day was calculated over an 8-day period, using the period from Day 13 to 20 (by which time all five species had largely stabilized at high rates of egg production; Fig. 2-2). Egg viability was calculated from the number of hatchlings divided by the number of eggs laid on Day 15 and on Day 20, and the mean egg fertility viability was

derived from these two data points. Total volume of eggs laid per day as also calculated from the number of eggs per day multiplied by egg volume as measured on Days 15 and 20.

To determine relationships between body size and reproductive parameters among species, regression analyses were conducted. The species means (as based on all experimental females combined) for the number of ovarioles, number of eggs laid per day, egg volume, egg viability, and total volume of eggs laid per day were regressed against the mean body weight of each species. All variables were log-transformed prior to analysis to facilitate interpretation from the perspective of body-mass specific rates (e.g., Brown and Sibly 2006). These analyses (and those described below) were performed by using SAS 9.1 (SAS institute, 2002–2003).

To analyze intraspecific variation, reproductive parameters were also regressed against body weight of individual females within species. Following Honek (1993), data from all five species were combined in analysis by standardizing within species for degree of deviation from the species mean for each variable. Thus, absolute values for body weight and reproductive parameters for each female of a given species were converted to percentage deviations from the mean value for that species [$\{(observed\ value - species\ mean)/species\ mean\}$; Honek 1993]. A two-way analysis of variance (ANOVA) was conducted first on the data to test whether slopes (of reproductive parameter x body weight) differed among species, followed by regression (based on a common slope for all five species) of standardized values of reproductive parameters against body weight of individual females. By this method, a single, general regression equation for all five lady beetle species combined was obtained to describe intraspecific

variation with body size for each reproductive parameter. These relationships could then be compared directly with those determined in the same manner by Honek (1993) based on a broad survey of published studies of insects in general.

Reproductive parameters of the similarly sized *C. septempunctata* and *C. transversoguttata* were compared in particular through further analysis, by taking into account intraspecific variation in body size. The number of ovarioles, number of eggs laid per day, egg volume, and the total volume of eggs laid per day were compared between the two species using analyses of co-variances (ANCOVA), with body weight as the covariate. Initially, a two-way ANOVA was conducted to test the assumption of homogeneity of slopes. Because the interaction of effects of species and wet weight was not significant ($P>0.05$ in all cases), an ANCOVA was then conducted by fitting a common slope for the covariate (body weight) for the two species to compare them for each reproductive parameter.

Results

Interspecific comparisons

The overwintered females of all five species laid very few eggs when first collected from alfalfa fields in early spring, but rapidly attained relatively stable, high rates of egg production after two weeks of provision with pea aphids in excess (Fig. 2-2). Among the five species, both the number of ovarioles and the number of eggs laid per day increased with body weight (Fig. 2-3a; log-log analysis for the number of ovarioles: $\log(y)=0.84(\pm 0.11)\log(x)+1.03$, $adjR^2=0.95$, $P=0.005$; Fig. 3b for the number of eggs per day: $\log(y)=1.69(\pm 0.21)\log(x)-2.71$, $adjR^2=0.95$, $P=0.004$). The

introduced *Coccinella septempunctata* had more ovarioles and laid more eggs per day than any of the four smaller, native species (Fig. 2-3). Individual egg volume, however, was not significantly related to body size among the five species (Fig. 2-3c; $r=0.62$, $P=0.25$). Egg viability was also unrelated to body size among species ($r=0.08$, $P=0.90$), and overall averaged $85.7\pm 0.01(\text{SE})\%$ for viable eggs laid by all five species combined. The total volume of eggs laid per day (i.e., number of eggs laid per day x mean egg volume) increased with body weight among the five species (Fig. 2-3d; $\log(y)=1.83(\pm 0.19)\log(x)-4.75$, $\text{adj}R^2=0.96$, $P=0.0022$).

Intraspecific comparisons

With values for reproductive parameters and body weight standardized as percentages of means within species, data from all five species were combined in analyses (Fig. 2-4). No significant interactions were found for the effects of species x body weight for any reproductive parameter (two-way ANOVA: $P>0.05$ in all cases). When a common slope (i.e., a single relationship) for all five species was fitted, the number of ovarioles increased significantly, but only slightly so, with an increase in body weight (Fig. 2-4a; $Y=0.16(\pm 0.05)X-0.0001$, $\text{adj}R^2=0.05$, $P=0.003$). The number of eggs laid per day by a female similarly increased, but did so more markedly, with an increase in body weight (Fig. 2-4b; $Y=0.75(\pm 0.09)X+0.0001$, $\text{adj}R^2=0.30$, $P<0.0001$). A relatively modest increase in egg volume also occurred with an increase in body weight (Fig. 2-4c; $Y=0.15(\pm 0.06)X+0.02$, $\text{adj}R^2=0.04$, $P=0.01$). Egg viability, however, did not increase with an increase in body size within species ($r=0.04$, $P=0.62$). Finally, the total volume of eggs laid per day increased strongly with an increase in body weight (Fig. 2-

4d; $Y=0.89(\pm 0.10)X-0.00001$, $adjR^2=0.35$, $P<0.0001$).

Reproductive comparison between of an invasive and a native species

Although females of *C. septempunctata* were larger on average than females of *C. transversoguttata* (Fig. 2-3, also see Fig. 2-1), there was broad overlap between the two species in body weight of individuals (Fig. 2-5). As revealed by ANCOVA, however, a female of *C. septempunctata* had more ovarioles than the same sized female of *C. transversoguttata* (Fig. 2-5a; species: $F_{1,80}=63.09$, $P<0.0001$, wet weight as covariate: $F_{1,80}=2.97$, $P=0.08$). Similarly, a female of *C. septempunctata* laid more eggs per day than the same sized female of *C. transversoguttata* (Fig. 2-5b; species: $F_{1,82}=18.17$, $P<0.0001$, wet weight: $F_{1,82}=31.81$, $P<0.0001$). In contrast, a female of *C. septempunctata* laid significantly smaller eggs than the same sized female of *C. transversoguttata* (Fig. 2-5c; species: $F_{1,82}=84.68$, $P<0.0001$, wet weight: $F_{1,82}=4.29$, $P=0.04$). Because of the trade-off between egg number and egg volume, no significant difference occurred in the total volume of eggs laid per day between the same sized females of *C. septempunctata* and *C. transversoguttata*; this parameter increased in the same fashion with body size in both species (Fig. 2-5d; species: $F_{1,82}=0.03$, $P=0.86$, wet weight: $F_{1,82}=43.52$, $P<0.0001$).

Discussion

The results of the present study indicate that the successful invader, *Coccinella septempunctata*, gains a reproductive advantage over native lady beetles from its larger body size when feeding on abundant prey. Associated with their larger size, females of

C. septempunctata have more ovarioles and can produce more eggs than can females of sympatric, native lady beetles. Females of *C. septempunctata* also lay a greater total volume of eggs per day than do smaller females of native species.

Previous studies also indicate that larger species have greater reproductive capacity than smaller species (e.g., Stewart et al. 1991b; Honek et al. 2008). This may favor large body size in habitats where food is abundant (Brown and Sibley 2006). Even so, larger species in general allocate less energy to reproduction on a mass-specific basis, with the interspecific relationship generally taking the form $y=ax^b$ with $b<1$; b is often estimated as equaling 0.75 (e.g., Millar 1977; Sibly and Calow 1986; Brown and Sibly 2006). Reiss (1985, 1989) notes, however, that estimates of b vary widely among studies of different groups of animals and have been reported as >1 in some studies.

In an earlier study of lady beetles, Stewart et al. (1991b) used literature values for eight aphidophagous species to estimate a nearly one to one interspecific relationship between increasing body weight and reproductive output (e.g., a doubling of body weight among species was associated with a doubling of reproductive output). This relationship also held between females of *C. septempunctata* and the much smaller *Propylea quatuordecimpunctata* L. that laid eggs when held without prey for a day after field collection (Honek et al. 2008). Stewart et al. (1991b) estimated b as 0.97 in a log-log regression of the biomass of eggs produced per day by a species (estimated as mean egg weight x number of eggs laid daily) against that species' mean adult weight. A similar relationship was obtained by log-log regression of reproductive biomass [defined as the number of ovarioles x egg weight] against adult weight (Stewart et al. 1991b). The sample of five, co-occurring lady beetles that consumed the same prey in

the present study yields $b=1.69$ and 1.83 (log-log regression) for the relationships between mean adult female weight of a species and total number and volume of eggs laid per day, respectively. Such high values of b based on a non-random, small group of species may not reflect broader patterns among aphidophagous lady beetles in general. These high b -values, however, make clear the considerable advantage in reproductive potential that the invasive *C. septempunctata* has over smaller, native lady beetles with which it shares alfalfa and the prey pea aphids therein.

Individuals within a species also vary considerably in size (Reiss 1985, 1989). Honek (1993) standardized data among numerous studies of intraspecific variation in diverse groups of insects by expressing values for individuals as percentages of the average for a species. The results indicated that an insect female's fecundity (i.e., her rate of production of offspring) increases in an almost one-to-one fashion with an increase in her body size (e.g., weight). Use of the same approach in the present study indicates no significant difference among the five lady beetle species in intraspecific variation in relative fecundity associated with relative female body size. For all five species combined, the intraspecific, positive slope for fecundity (number of eggs laid per day) regressed against body weight was 0.75 . This was somewhat lower than the slope of 0.95 estimated by Honek (1993) for insects as a whole as well as the slope of 0.93 reported by Dixon and Guo (1993) for the relationship between egg cluster size (approximately equal to number of eggs per day) and body weight for females of *C. septempunctata* when provided excess prey (pea aphids). When an adjustment is made for fecundity by multiplying egg number by mean egg volume for each species in the present study, however, a very similar slope of 0.89 is obtained for the volume of eggs

laid per day as previously reported, an increasing function of female body weight (Millar 1977; Sibly and Calow 1986; Brown and Sibly 2006).

Results were similar for the relationship of female body weight and fecundity in the present study when the latter was expressed either as number of eggs laid per day or as volume of eggs laid per day. This reflects relatively little mean egg volume variation among conspecific females and only a weak relationship with their body size (slope=0.15 for the relationship when data were expressed as percentages of species means). Honek (1993) similarly analyzed two published data sets (for Chrysomelid beetles and butterflies) and also obtained only slight, positive slopes (0.2–0.3) for intraspecific relationships between the size of a female and the size of her eggs. The weakness of such relationships supports the conclusion that egg sizes of lady beetles vary little within species and can be regarded for the most part as a species specific characteristic (Stewart et al. 1991a,b; Dixon and Guo 1993).

Given the strong relationship between body size and fecundity within species, intraspecific variation in body size of *C. septempunctata* and the similarly large native congener, *C. transversoguttata*, broadly overlapped when comparing the reproductive potentials of these two species. On average, *C. septempunctata* was slightly larger than *C. transversoguttata*, and correspondingly the former species on average had more ovarioles, and laid more eggs and total volume of eggs per day. There was broad overlap in body size, however, between *C. septempunctata* and *C. transversoguttata*. Therefore, reproductive parameters were compared between the two species while controlling for intraspecific variation in body size.

Females of *C. septempunctata* had more ovarioles than did females of *C.*

transversoguttata of the same size. As the functional units of the insect ovary, ovarioles determine the potential reproductive output of a female (Stewart et al. 1991a,b; Dixon and Guo 1993; Hodin and Riddiford 2000). From a survey of eleven species, Stewart et al. (1991a) found that the mean number of eggs laid in a clutch is equal on average to half the median number of ovarioles for a given species of aphidophagous lady beetle. Within a species, the number of ovarioles varies with female size. In expressing variables as percentages of means for a wide array of insects, Honek (1993) obtained a positive slope of 0.81 for the intraspecific increase in number of ovarioles with increasing female weight. For *C. septempunctata* reared as larvae on varying numbers of aphids, Dixon and Guo (1993) reported a slope of 1.3 for ovariole number regressed against adult female size. In contrast, a much weaker intraspecific relationship between ovariole number and female weight (slope=0.16) occurred in the analysis [patterned after Honek (1993)] of combined data for the five lady beetles studied here. In general, the number of ovarioles in an adult female insect depends both on genetics and on food availability during larval development (e.g., Rhamhalinghan 1985; Honek 1993; Starmer et al. 1998; Wayne and Mackay 1998; Hodin and Riddiford 2000), with important influence from such factors as epistatic genetic effects, genotype x environment interactions, and maternal effects (Starmer et al. 1998; Wayne and Mackay 1998). The experiments presented here were conducted with field-collected adult females of unknown history and genotype. This may explain why so little variation in ovariole number was accounted for by body size differences among these females. Osawa (2005) also found little relationship between ovariole number and body size of field-collected females of the lady beetle, *Harmonia axyridis* Pallas. Although larger

body size within species was not strongly related to a larger number of ovarioles in the present study, females of larger body size nonetheless may be able to mature more eggs simultaneously in a larger number of fully developed ovarioles, given their larger physical capacity to accommodate enlarged gonads (Honek 1993). Thus, larger females may have been more able to realize their reproductive potential as set by the number of ovarioles (Stewart et al. 1991a), even though they differed relatively little from smaller females on average in the number of ovarioles that they possessed.

Coccinella septempunctata laid more eggs per day than *C. transversoguttata* of the same size, but interestingly, also laid smaller eggs. Thus, comparison of *C. septempunctata* and *C. transversoguttata* reveals a trade-off between egg number and egg size (e.g., Smith and Fretwell 1974). A similar trade-off is apparent as well for the smaller congeneric pair of species, *Hippodamia convergens* and *H. quinquesignata*. Because of the trade-offs between egg number and egg size for these two pairs of similarly sized species (*C. septempunctata* vs *C. transversoguttata*, and *H. convergens* vs *H. quinquesignata*), a significant interspecific relationship between egg volume and body size was not found in this study.

The absence of a significant, positive interspecific relationship between female body size and egg size most likely reflects that comparisons in the present study were limited to a small group of five species that co-inhabit alfalfa. Previous literature-based studies of lady beetles indicate that such a relationship holds more generally among species in this group of insects. Stewart et al. (1991b) report from literature values for eight species of aphidophagous lady beetles that the log of egg weight is positively related to log adult weight, but with a slope less than one (0.63). Expanding on this

literature survey to include additional species, Dixon and Hemptinne (2001) confirmed that females of larger species (of both aphidophagous and coccidophagous lady beetles) lay larger eggs (in absolute dimensions) than do females of small species. Larger species, however, also lay smaller eggs relative to their size (i.e., in relative dimensions), and hence like other animals, appear to invest relatively less in individual offspring (Dixon and Hemptinne 2001; Honek et al. 2008). Furthermore, Stewart et al. (1991b) reported an inverse interspecific relationship in lady beetles between the number of ovarioles per unit body mass and the size of individual eggs, as had been reported also for aphids (Dixon 1987) and aphidophagous Syrphidae (Gilbert 1990). This relationship is apparent in the present study as well, as females of *C. septempunctata* both had more ovarioles and produced more and smaller eggs than similarly sized females of *C. transversoguttata*. A similar contrast in reproductive patterns was apparent between the similarly sized females of *H. convergens* and *H. quinquesignata*.

The possible adaptive significance of egg size differences that accompany differences in rates of egg production between species is intriguing. Of potential importance are effects of egg size on success and rates of larval development. Dixon and colleagues (Stewart et al. 1991 a,b; Dixon and Guo 1993; Dixon 2000) have hypothesized that minimum egg size for an aphidophagous lady beetle is set by the challenge for the newly hatched larva of finding and overcoming its active prey. Using values from the literature for six species of aphidophagous lady beetles, Stewart et al. (1991b) confirmed the prediction that, in general, species with relatively small eggs (i.e., low egg to adult weight) have longer development times than species with

relatively large eggs. But such may not be the case for larvae of *C. septempunctata* and *C. transversoguttata* that develop in alfalfa fields with readily available pea aphid prey. When reared on pea aphids and green peach aphids (*Myzus persicae* [Sulzer]) at five constant temperatures, larvae of *C. septempunctata* consistently had shorter developmental times than larvae of *C. transversoguttata* (Obrycki and Tauber 1981).

When egg number and egg size are combined to calculate total egg volume laid per day, the larger *C. septempunctata* produces more than the smaller *C. transversoguttata* on average. But when body size is factored in, total egg volume varies with body size in the same fashion for these two species. Thus, the greater rate at which eggs are laid by females of *C. septempunctata* versus *C. transversoguttata* comes from the former species' larger average size and from its partitioning of a given total volume of eggs per day (as determined by body size) into a larger number of smaller eggs.

Overall, the data presented here suggest that *C. septempunctata* can succeed in producing the largest number of offspring per unit time in alfalfa fields with abundant aphids. This may help to explain its prevalence over native lady beetle species in this habitat. The successful invasion of North America in general by *C. septempunctata* has been attributed to its life history strategy as an "r-selected", reproductive opportunist (Hodek and Honek 1996; Evans 2000; Hodek and Michaud 2008). The results of the present study are consistent with this hypothesis. Whether additional factors (e.g., intraguild predation) may also be important in accounting for the rapid rise to dominance by *C. septempunctata* in alfalfa fields of western North America remains to be determined. That additional factors may also play a role, however, is suggested by the

observation that the difference in relative abundances of *C. septempunctata* and *C. transversoguttata* in these fields is even greater than the difference documented here in their reproductive potential at maximum rates of prey consumption.

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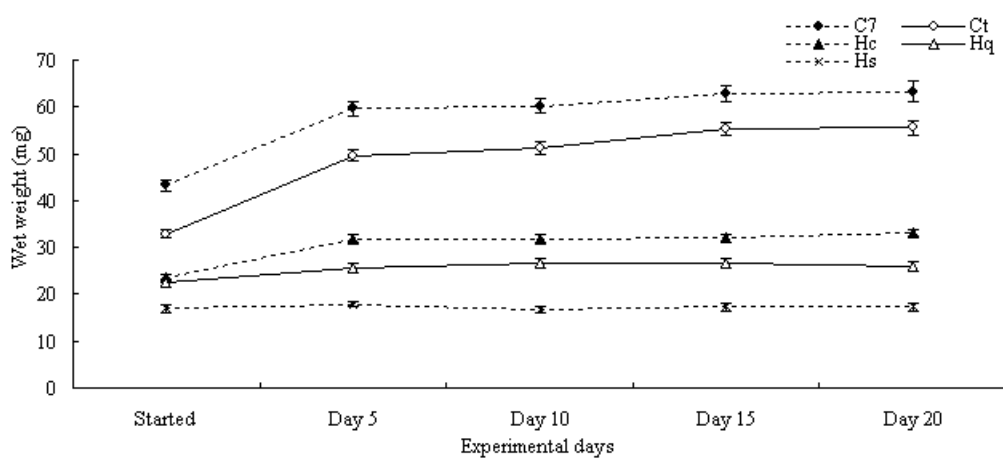


Fig. 2-1 Mean wet weights (\pm SE) of females *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), *Hippodamia convergens* (Hc), *H. quinquesignata* (Hq), and *H. sinuata* (Hs) upon being collected from alfalfa fields, and on subsequent days with provision of excess prey (pea aphids).

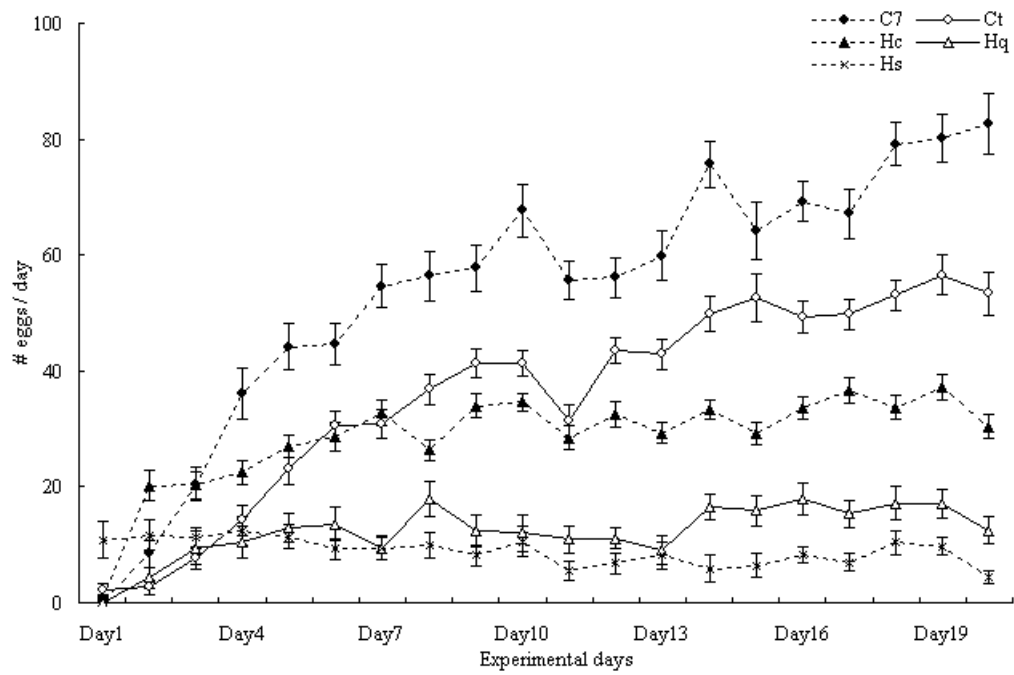


Fig. 2-2 The mean number of eggs (\pm SE) laid by females of *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), *Hippodamia convergens* (Hc), *H. quinquesignata* (Hq), and *H. sinuata* (Hs) on successive days with provision of excess prey (pea aphids).

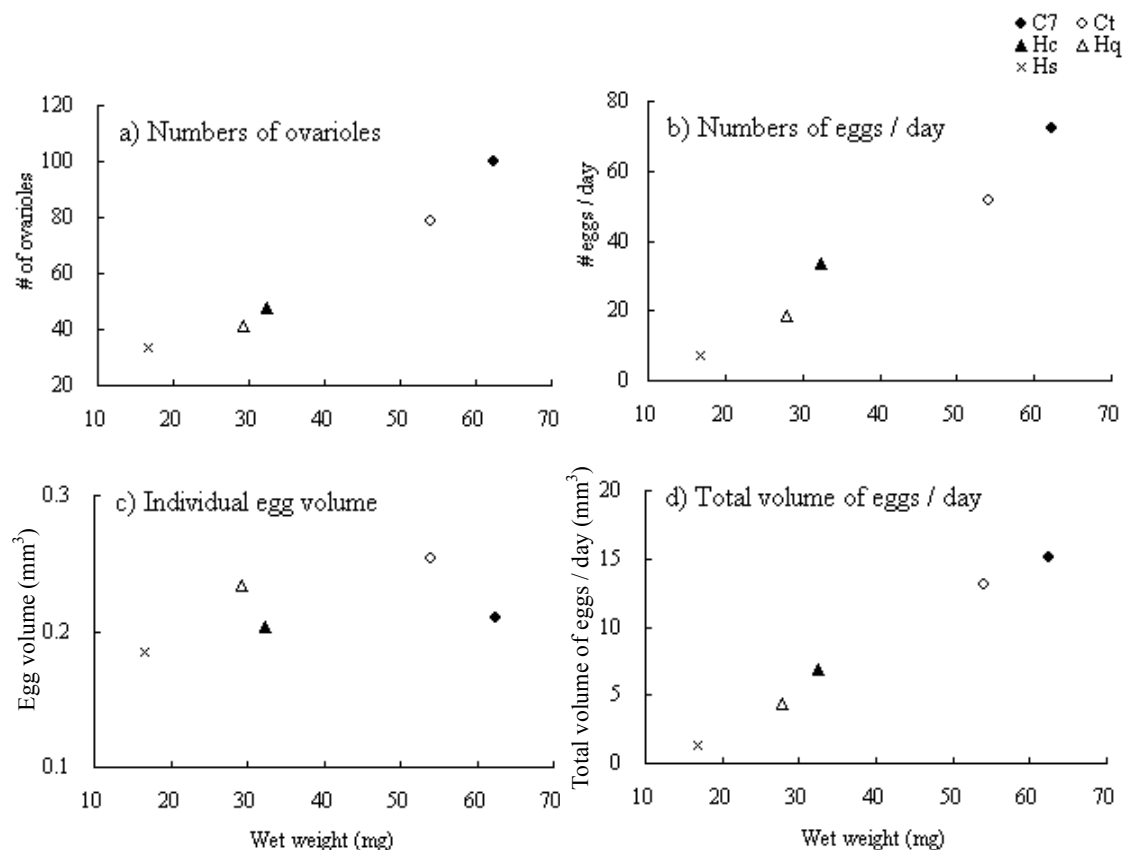


Fig. 2-3 Interspecific relationships between mean adult female body size (wet weight in mg) of individual species when field-collected females of *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), *Hippodamia convergens* (Hc), *H. quinquesignata* (Hq), and *H. sinuata* (Hs) were provided with excess prey (pea aphids), and species means for (a) ovariole number, (b) egg production per day, (c) egg volume (i.e., size of individual eggs), and (d) total volume of eggs laid per day.

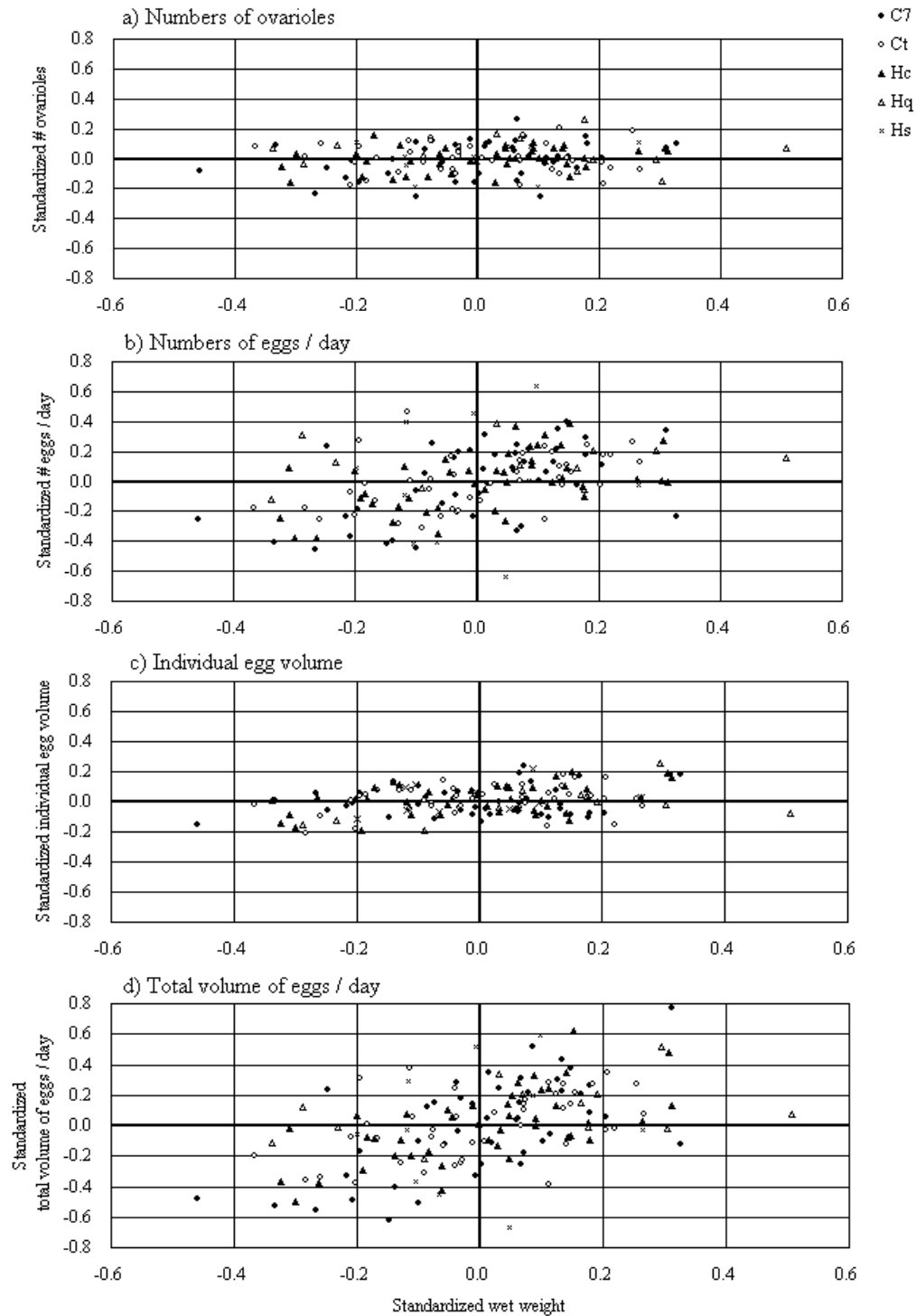


Fig. 2-4 Intraspecific relationships for data standardized (as percentages of the mean) among species following Honek (1993), between body size (wet weight in mg) of individual females of five lady beetle species of *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), *Hippodamia convergens* (Hc), *H. quinquesignata* (Hq), and *H. sinuata* (Hs), and (a) numbers of ovariole number, (b) number of eggs per day, (c) individual egg volume, and (d) total volume of eggs per day.

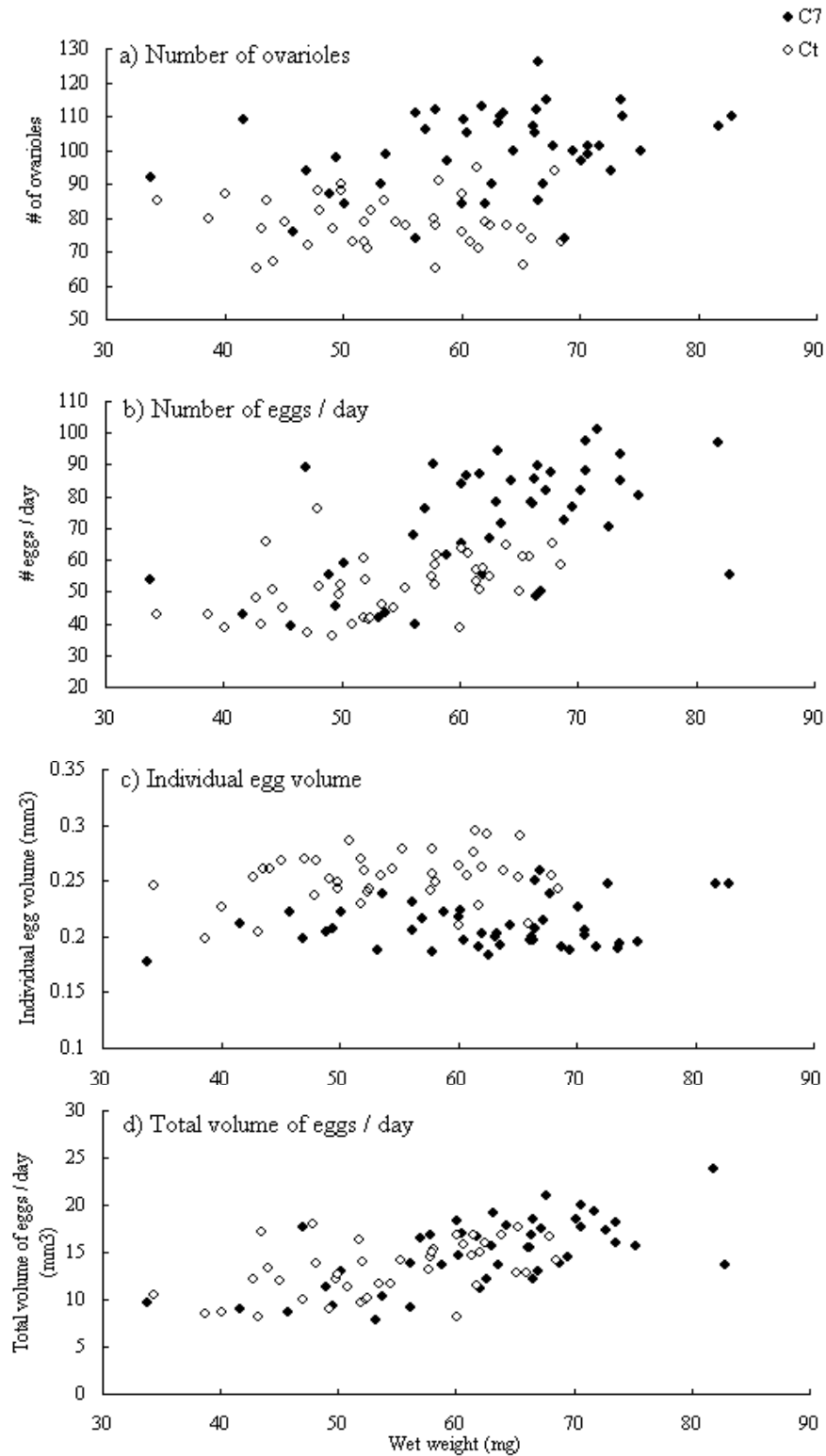


Fig. 2-5 Relationships between body size (wet weight in mg) and (a) number of ovarioles, (b) number of eggs per day, (c) individual egg volume, and (d) total volume of eggs per day of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct).

CHAPTER 3

REPRODUCTIVE COMPARISON BETWEEN AN INVASIVE AND A NATIVE
LADY BEETLE IN ALFALFA FIELDS IN RESPONSE TO
PREY POPULATION DYNAMICS

Abstract Non-native predatory lady beetles (or ladybirds; Coleoptera: Coccinellidae) have been introduced to North America in recent decades raising concerns of adverse impact on native species, especially native lady beetles. In northern Utah, *Coccinella septempunctata* first appeared in 1991, and is now predominant among lady beetles in alfalfa fields. The suitability of alfalfa as habitat for lady beetles is examined by comparing reproduction of overwintering females of *C. septempunctata* and the native, similarly sized *C. transversoguttata*, which is much less abundant now in this habitat than is the introduced *C. septempunctata*. The population dynamics of lady beetles and their aphid prey and the seasonal egg laying patterns of the predators were examined in alfalfa hay fields near Logan, Utah, over a 3-year period (2004–2006). Populations of aphids increased from late April through the spring and early summer and then decreased after the first cutting of hay and remained low thereafter. Adult lady beetles were collected weekly from these fields, were weighed, and then held under constant condition for 48 hours and the number of eggs laid was determined. A significant difference in the timing and amount of egg production was found between *C. septempunctata* and *C. transversoguttata*. Females of *C. septempunctata* reproduced eggs sooner and laid more eggs than *C. transversoguttata* females even at low prey density. Such early reproductive activity and high fecundity in the invasive *C. septempunctata* was observed consistently among fields and years. *C. septempunctata* females were more successful in approaching

their maximum body weights and reproductive output than were females of native *C. transversoguttata* when lady beetle performance was compared in the laboratory under ideal conditions. The superior reproductive success of the invader *C. septempunctata* suggests a mechanism by which *C. septempunctata* has become so successfully established in North America.

Introduction

Because of their many disruptive effects on ecosystems worldwide, invasive species have attracted much attention and concern in recent years (e.g., Mack et al. 2000). Of major interest are factors and processes that promote species invasions of new geographic areas. In at least some cases, introduced species may succeed initially in establishing in especially suitable habitats and then spread from these core habitats to invade diverse additional habitats. In the case of insects, for example, such core habitats may often be agricultural lands with favorable physical conditions (e.g., as provided by irrigation) and abundant food resources, including both host plants for herbivores and pest insects for natural enemies (e.g., Rand and Louda 2006; Rand et al. 2006). In particular instances, insect natural enemies are often introduced purposefully to agricultural crops in new geographic areas in the practice of classical biological control. In other cases, they establish themselves in new geographic regions without intentional human assistance and grow to large numbers in agricultural crops. Thus, these invaders may often be characterized by initial establishment in agricultural habitats (source habitats), with subsequent spread to other habitats in local communities.

This general pattern of invasion may apply to the recent, high profile invasive

spread in North America by species of lady beetles. These invasions have raised widespread concern regarding potentially adverse effects on native North American lady beetles (Howarth 1991; Simberloff and Stiling 1996; Elliott et al. 1996; Louda et al. 2003). Here, the case of *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) and its recent invasion of the Intermountain West of North America (e.g., Evans 1991, 2004) is considered. In the generally arid landscape of the Intermountain West, irrigated alfalfa often supports high aphid populations and provides a favorable, mesic environment for aphidophagous lady beetles. Many native North American lady beetle species moved into alfalfa following its introduction to the region in the nineteenth century (Evans 2000). Since its arrival and initial establishment in the Intermountain West in the early 1990s, *C. septempunctata* has consistently maintained high populations in alfalfa fields while at the same time the densities of native lady beetles have declined (Evans 2004). The high numbers of *C. septempunctata* in fields of the introduced alfalfa, with abundant populations especially of the introduced pea aphid (*Acyrtosiphon pisum*, Hemiptera: Aphididae), reflect furthermore that this lady beetle readily and routinely attacks this prey in this same habitat in the predator's native (Palearctic) range (e.g., Hodek and Honek 1996). These observations suggest that alfalfa in North America may serve as a source habitat promoting regionally high numbers of *C. septempunctata*.

To address a key aspect of this general hypothesis, the more specific hypothesis is tested here that alfalfa in the Intermountain West is particularly favorable as a habitat for *C. septempunctata*, as is suggested by its numerical dominance over native North American lady beetles in alfalfa. As a measure of habitat suitability, the ability of lady beetles to reproduce in alfalfa fields is evaluated. Such ability is compared between

overwintering females of *C. septempunctata* and the native and similarly sized *C. transversoguttata*, which is much less abundant now in this habitat than is the introduced *C. septempunctata* (Evans 2004). In a related study conducted under laboratory conditions (Chapter 1), *C. septempunctata* females produced more and individually smaller eggs per day than *C. transversoguttata* females when pea aphids were provided in excess. Whether *C. septempunctata* females also achieve greater fecundity than *C. transversoguttata* females under natural conditions in alfalfa fields was examined here. Furthermore, whether *C. septempunctata* females more nearly achieve their reproductive potential (as measured in the laboratory) than do females of *C. transversoguttata* was also tested. The seasonal reproductive patterns of both lady beetle species were also compared with the temporal dynamics of aphid populations in alfalfa. Such comparison was made in part with reference to the egg window hypothesis (Hemptinne et al. 1992; Kindlmann and Dixon 1993; Dixon 1997, 2000) and its key prediction that aphidophagous lady beetles lay most eggs prior to the attainment of peak numbers in local populations of their aphid prey.

Materials and Methods

Aphid and lady beetle sampling

Lady beetles and pea aphids were sampled each week in alfalfa fields of northern Utah, near Logan in Cache County, from mid April through early July in 2004 and 2005. Two fields were sampled in each year. In 2004, these fields were located at the Utah State University Animal Science and Caine Dairy farms. In 2005, fields were located at the USU Cache Junction and Wellsville Farms. Additional sampling was conducted in an

alfalfa field at the Wellsville Farm from May 15 until September 4 in 2006 to examine the reproductive activity of lady beetles in late summer and fall. The alfalfa in these fields was cut for hay. The first cutting occurred on June 2 in 2004 (both fields), June 24 in 2005 (both fields), and June 5 in 2006. In 2006, the field was cut again in mid July. At each cutting, a strip (15 x 50–60 m) of alfalfa was left uncut in each field. This strip was sampled thereafter for the next 2–3 weeks until the cut alfalfa had regrown to 30cm height and could once again be sampled for both lady beetles and pea aphids.

Densities of pea aphids were determined weekly by sampling alfalfa stems. Twenty alfalfa stems were sampled at each of five randomly selected locations at each census in each field in 2004. Ten alfalfa stems were sampled at each of twenty-five random locations in each field in 2005 and 2006. Immediately upon collection in the field, the stems were shaken within a white bucket (30cm in diameter x 35cm in height). The dislodged pea aphids were counted (previous use of this technique was shown to dislodge more than 97% of the aphids from the stems; E. W. Evans, personal observations).

To attract invasive and native lady beetles to alfalfa fields for collection, 10m² plots within each field were sprayed with a sugar solution (15% sucrose) each week during the growing season (e.g., Evans and England 1996). Consumption of the sugar does not promote egg production in *C. septempunctata* and *C. transversoguttata*, which instead is stimulated by aphid consumption (Richards and Evans 1998). Lady beetles were collected from plots during the next 2–5 days following sugar spraying, and brought to the lab immediately. The females' wet weights were measured, and then each female was placed individually in a Petri dish (5.5cm in diameter, 1.5cm in height) with one drop

of sugar water (15% sucrose) on the lid. Dishes were kept in an incubator (22°C, 16L:8D) for 48 h. The number of eggs laid in the dish was counted at 24 and 48 h. In those instances in which females cannibalized their own eggs, the residue from each cannibalized egg (a spot where the egg had been attached to the substrate) was noted and included in counts of eggs produced. Maintaining females in this fashion provided an estimate of the number of mature or nearly mature eggs that a female had at the time of collection. Females held without prey will proceed to lay such eggs (rather than resorb them) over a 48-h period upon removal from prey (Richards and Evans 1998; Honek et al. 2008; Chapter 5).

Sweep-net sampling (ten sweeps x ten samples) was conducted in 2004 to examine the number of lady beetle larvae occurring in the alfalfa fields after the first cut (i.e., when larvae became abundant enough to sample) from June 11 to July 14. Sampling was initiated near the center of the fields, with successive samples taken along a transect towards the edge of the field. All collected larvae were placed in a vial, and immediately brought back to the lab to distinguish species. To confirm species identity, each larva was kept individually in a Petri dish with prey (pea aphids) until it molted into an adult.

Visual censuses were also conducted in 2004 to find pupae in fields. Censuses of 60 person-minutes were conducted along the edge of each field (where pupae could be found most readily) upon completion of sweep-net sampling. Pupae were placed in a vial and kept individually in a Petri dish in the lab. The species identity of each pupa was determined upon at emergence of adults.

In 2005, visual censuses were conducted weekly to examine the population dynamics of adult predatory lady beetles in fields. These censuses were conducted at least

5m from sucrose plots. As with sweep sampling in 2004, each census (of 60 person-minutes) began in the center of the field and continued along a straight transect toward the edge of the field. Adult lady beetles were collected from the alfalfa foliage, placed in a vial, and identified to species thereafter in the lab.

The tendencies of successive generations of *C. septempunctata* and *C. transversoguttata* over the growing season to initiate reproduction (versus enter reproductive diapause) in northern Utah were assessed by rearing successive generations in a greenhouse (with natural day length) throughout the summer of 2006. Overwintered lady beetles were collected from alfalfa fields in late May, were paired and allowed to mate in the greenhouse. Each pair was placed in a Petri dish and provided with excess prey (pea aphids, reared on fava beans, *Vicia faba* L. [Fabaceae] in the greenhouse). Eggs laid by the overwintered females were selected at random in early to mid June, and reared to obtain first generation adults (the eggs were taken from 20 overwintered females). From these first generation adults (which emerged beginning July 12), 40 pairs of *C. septempunctata* and 30 pairs of *C. transversoguttata* were maintained to score for reproductive activity (versus diapause) and to obtain the next (second) generation of eggs. Pairs were examined from July 12 for the next three weeks to determine whether or not females laid eggs (i.e., became reproductively active). Eggs were selected at random from reproductively active females, and were reared in the greenhouse to obtain second generation adults. From these second generation adults (which emerged beginning August 15), 26 pairs of *C. septempunctata* and 14 pairs of *C. transversotuttata* were paired and held as described above. Females were scored for reproductive activity for 2 weeks beginning August 15. From their eggs, adults of the third generation of the two

species were paired after beginning to emerge on September 30. This third generation of adults (20 pairs of *C. septempunctata* and 21 pairs of *C. transversoguttata*) was scored for a month for reproductive activity, beginning September 30. Prior to pairing of third generation adults, all lady beetles were moved in mid-September from the greenhouse to a laboratory room with natural lighting, and with ambient temperature at approximately 20°C.

Data analyses

In both 2004 and 2005, overwintered lady beetles were especially active in producing and laying eggs in alfalfa for a period of about one month during spring through early summer. For analyses, the data were divided therefore into three periods of the season: an early period (when most lady beetles were still inactive reproductively), a peak period (the mid, four-week period during which most lady beetle reproduction occurred), and a late period (occurring after the first cut of alfalfa and when surviving females from the overwintering generation were joined by newly molted adults from the new summer generation). For further discrimination, the peak period (with high reproductive activity) was divided into three parts: early peak (1 week), mid peak (2 weeks), and late peak (1 week) (Table 3-1).

Differences in weather conditions between years (Table 3-2) resulted in different seasonal timing of lady beetle reproductive activity between 2004 and 2005. In particular, the spring of 2005 was cooler and wetter than the spring of 2004, and correspondingly lady beetle reproductive activity occurred later in 2005 (Table 3-1; also see Fig. 3-5).

To compare among years, the first week (“week 1”) for the field studies in each

year was considered to be the third week of April (beginning April 19 in 2004, 18 in 2005 and 17 in 2006). This is the week that sampling of alfalfa fields began in 2004, whereas the first week of sampling in the cooler spring of 2005 was week 4 (May 9). As indicated in Table 1, data were pooled from individual females (collected in the field and evaluated in the lab) according to weeks of collection that correspond to the seasonal periods (and sub-periods) of reproductive activity as described above.

The reproductive activity of females of *C. septempunctata* and *C. transversoguttata* was compared during the different periods of the season by conducting three sets of analyses. First, a comparison was made of the percentages of field collected females that were gravid when collected and produced eggs when held subsequently for 48 h in the laboratory. This comparison was made for females of the two species collected during the early and late periods (as defined above), and during each of the three peak reproductive sub-periods (early, mid, and late). Because seasonal reproductive patterns (including seasonal percentages of females that were gravid) were very similar overall between the two fields sampled in a given year, these patterns were compared for *C. septempunctata* and *C. transversoguttata* by pooling data from two fields. To account for small sample sizes (particularly of *C. transversoguttata* females) in comparisons, an exact test (Monte Carlo Estimate, $n=10000$) was used for the analyses.

The reproductive activity of *C. septempunctata* and *C. transversoguttata* was also compared for the number of eggs laid during 48 h after collection. For this comparison, only those females that laid eggs (one or more) were included. A two-way analysis of variance (ANOVA) was used to compare between the two species and the two years, the number of eggs (transformed by square root) laid. The analysis was focused on the mid

peak time period each year (Week 6 and 7 in 2004, Week 8 and 9 in 2005), when reproductive activity overall was at its height.

Rates of egg production of gravid females of *C. septempunctata* and *C. transversoguttata* collected from the field at mid peak were compared to the potential rates that females could achieve under ideal conditions. Thus, the rates of field collected gravid females were estimated as the number of eggs laid in the 48 h following collection divided by the mean number of eggs laid by a reproductively active, overwintered female when maintained in the laboratory and provided with excess pea aphids. As the estimate for this latter number, the mean number of eggs (162.9 for *C. septempunctata*, and 116.3 for *C. transversoguttata*) as determined on days 19–20 in the laboratory experiment described in Chapter 2 was used. Data were transformed by square root, and then analyzed by two-way ANOVA (species, year, species x year).

Three-way ANOVA (species x year x period) were used to compare changes in wet weight of all females of *C. septempunctata* and *C. transversoguttata* collected in alfalfa fields between early and peak periods. Wet weights of females collected in alfalfa fields in 2004 and 2005 during the peak period were also compared after dividing them by the mean wet weight of a fully fed female (estimated as 63.0 mg for *C. septempunctata* and 55.4 mg for *C. transversoguttata*, which were the average wet weights at day 15 and 20 for females provided with excess numbers of pea aphids in the laboratory [Chapter 2]) by two-way ANOVA.

Tendencies of successive generations of *C. septempunctata* and *C. transversoguttata* over the growing season to initiate reproduction (versus enter reproductive diapause), as assessed in the greenhouse, were compared between the two

species by χ^2 test. All analyses were performed by using SAS 9.1 (SAS institute 2002–2003).

Results

Pea aphid populations

The density of pea aphids in alfalfa fields peaked in late May to early June in 2004 and in mid to late June in 2005 (Fig. 3-1). Because the early spring was cold in 2005 (Table 3-2), the aphid population increased more slowly than in 2004. After the alfalfa was cut for the first harvest (in early June in 2004, and in late June in 2005), aphid populations declined greatly in uncut and cut sections, with declines in uncut sections reflecting increasing daytime temperatures and likely also increasing damage from alfalfa weevil larvae. Aphid populations remained very low thereafter as high daytime temperatures prevailed in late June and July.

Lady beetle populations

In 2004, numbers of adult lady beetles were not quantified by visual census, but adults of *Coccinella septempunctata* were observed to be the most abundant lady beetles present in the alfalfa fields, and they were collected in greatest numbers from sugar plots throughout the season. In 2005, weekly visual censusing of adults revealed that *C. septempunctata* was again the most common species present throughout the spring and early summer in the alfalfa fields, including both in early spring (weeks 4–6) and after the first cut, when many adults were newly molted individuals of the first generation (Fig. 3-2).

Wet weights of field-collected lady beetles

The wet weight of field-collected females of *C. septempunctata* was greater than that of *C. transversoguttata* throughout the experimental period (Fig. 3-3). Three-way ANOVA yielded significant effects, including a significant three-way interaction of species x year x period (Table 3-3 and Fig. 3-4). Females of both species gained weight from the early to peak period, but the absolute gain in weight (mg of weight gain) was larger for *C. septempunctata* females (Fig. 3-4). This was especially true in 2005, when cold weather during the early period was associated with low aphid populations and lower mean weights of females of both species than was the case in early 2004.

Field collected females of both species during the peak period in 2004 and 2005 weighed less than females provided with excess aphids in the lab (see Chapter 2). Mean weights of field collected females of *C. septempunctata* were $79\pm 1\%$ of maximum potential weight in both 2004 and 2005, and mean weights of females of *C. transversoguttata* were $73\pm 2\%$ and $75\pm 1\%$ of maximum weight in 2004 and 2005, respectively. These field-attained percentages of the maximum weights for females of the two species were significantly higher for *C. septempunctata* females than for *C. transversoguttata* females (two-way ANOVA: effect of species $F_{1,620}=13.38$, $P=0.0003$; effect of year $F_{1,620}=0.29$, $P=0.59$; interaction of species x year $F_{1,620}=0.49$, $P=0.49$).

Reproduction of lady beetles

In general, the seasonal reproductive patterns of *C. septempunctata* and *C. transversoguttata* were similar in both fields for a given year (Fig. 3-5). Females of both *C. septempunctata* and *C. transversoguttata* produced few eggs during the early period.

Egg production rose rapidly during the peak period, only to fall again to low levels in the late period. Throughout the season, *C. septempunctata* females generally produced more eggs during any given week than did *C. transversoguttata* females. This general pattern was analyzed further by examining both the percentage of females laying eggs, and the number of eggs laid by reproductive females.

In 2004, *C. septempunctata* females on average began to lay eggs earlier during the spring than did females of *C. transversoguttata* (Fig. 3-6a). More than 20% of *C. septempunctata* females, versus only 6.5% of *C. transversoguttata* females, laid eggs upon collection during the early period (weeks 1–4). Throughout the peak period (weeks 5–8), approximately 80% of *C. septempunctata* females laid eggs. In contrast, the percentage of *C. transversoguttata* that laid eggs increased more gradually, and did not reach its maximum (at less than 60%) until late in the peak period (week 8). Most of the lady beetles collected from mid-June on (weeks 9–14) were new adults (recognized by their soft and brightly colored exoskeletons) that did not lay eggs.

During the cool spring of 2005, very few females (less than 3%) of both *C. septempunctata* and *C. transversoguttata* laid eggs during the early period (weeks 4–6) (Fig. 3-6b). However, as the temperatures warmed by week 7, once again a much greater percentage of *C. septempunctata* females (50%) than of *C. transversoguttata* females (12%) became reproductively active and laid eggs. A greater percentage of *C. septempunctata* females continued to lay eggs thereafter (including during the late period); however, there were not significant differences between *C. septempunctata* and *C. transversoguttata* during the late peak and late period (week 10, and weeks 11–14, Fig. 3-6b).

Consistently among fields and between years, *C. septempunctata* females laid more eggs than *C. transversoguttata* females (two-way ANOVA: effect of species $F_{1,398}=34.47$, $P<0.0001$, interaction of year x species $F_{1,398}=1.15$, $P=0.18$). Overall, the average egg production of both species was greater in 2004 than in 2005 (effect of year $F_{1,398}=96.87$, $P<0.0001$).

In both years, the ratio of egg production of field-collected females to mean egg production of females maintained on an excess diet of pea aphids in the laboratory was greater for *C. septempunctata* than for *C. transversoguttata* during the mid-peak period (2004: 0.33 ± 0.01 versus 0.26 ± 0.04 , 2005: 0.26 ± 0.01 versus 0.16 ± 0.02 , two-way ANOVA: effect of species $F_{1,398}=6.04$, $P=0.014$, effect of interaction of year x species $F_{1,398}=1.09$, $P=0.29$). For both species, the field rate of reproduction more closely matched the maximum potential rate in 2004 than in 2005 (effect of year $F_{1,398}=35.37$, $P<0.0001$).

In both alfalfa fields, many more *C. septempunctata* than *C. transversoguttata* eggs were produced throughout the season (estimated by the total number of eggs laid each week by the populations of *C. septempunctata* and *C. transversoguttata* females during 2005; see Methods). Both species produced the largest numbers of eggs before the aphid populations peaked in size (Fig. 3-8).

Most larvae (89%) and pupae (98%) that occurred in the Animal Science and Caine Dairy alfalfa fields belonged to *C. septempunctata* (Table 3-4). A few individuals of other species (*Hippodamia convergens*, *H. quinquesignata*, and *H. sinuata*) were collected. Interestingly, no larvae or pupae of *C. transversoguttata* occurred in our samples.

Late season patterns in 2006

Overall, patterns in spring and early summer were similar to those observed in 2004 and 2005. Thus, females of *C. septempunctata* weighed more, and reproduced more in the first part of the season, than females of *C. transversoguttata* (Fig. 3-9b,c). From mid summer on (i.e., from week 10 on), the pea aphid population was very small (Fig. 3-9a), and none of the *C. septempunctata* and *C. transversoguttata* females collected produced eggs (Fig. 3-9c).

Number of lady beetle generations per year

When reared and maintained under natural day length in the greenhouse during mid summer, 92% of first generation females of *C. transversoguttata*, but only 45% of females of *C. septempunctata* were reproductively active (Table 3-5; $\chi^2=15.12$, $P=0.0001$). Only 8 and 14% of second generation *C. septempunctata* and *C. transversoguttata* females, respectively, laid eggs. Only 5% of third generation females of both species were reproductively active (Table 3-5; comparisons between species: 2nd generation: $\chi^2=0.43$, $P=0.51$; 3rd generation: $\chi^2=0.0013$, $P=0.97$). Furthermore, eggs produced by reproductively active females in the third generation did not hatch. Thus, although only overwintered females of these two lady beetle species were reproductively active in alfalfa fields, it appears that the two species have the potential to complete as many as three (partial) generations each year. Nonetheless, *C. septempunctata* females have a stronger tendency than *C. transversoguttata* females to enter reproductive diapause as first generation adults.

Discussion

General patterns of lady beetle reproduction in alfalfa fields

Females of *Coccinella septempunctata* and *C. transversoguttata* produced few eggs early in the spring when pea aphid populations in alfalfa fields were low. They produced more eggs as aphid populations increased thereafter and reached peak reproduction during mid May to early June in 2004, and late May to mid June in 2005. Cooler weather in 2005 appeared to slow aphid population growth and the associated onset of reproductive activity of the lady beetles. The overall lower rates of lady beetles' egg production in 2005 compared to 2004 was also likely influenced by cooler spring weather in 2005.

The first cutting of alfalfa occurred in early June 2004 and in mid June 2005. With high daytime temperatures thereafter, pea aphids became less abundant in alfalfa fields and lady beetle reproductive activity slowed. Larvae and pupae of the first generation of lady beetles (i.e., the offspring of overwintered individuals) were found in fields during June. Newly molted adults were collected in the second half of June. Field censusing in 2006 documented that, as observed also (but not quantified) in 2004 and 2005, very low pea aphid numbers and very little egg production by lady beetles occurred after mid summer. Thus, the reproductive activity of both species of lady beetles in alfalfa fields occurred primarily in spring and early summer (May to early June).

Analysis of the seasonal patterns of pea aphid population dynamics and lady beetle reproductive activity indicates that females of both *C. septempunctata* and *C. transversoguttata* collectively (i.e., as populations) produced most eggs in 2005 before

pea aphid numbers peaked before the first cutting of alfalfa (a similar analysis could not be made for 2004 because adult lady beetle abundances were not quantified). Such a predator-prey temporal relationship is predicted by the egg window hypothesis for aphidophagous lady beetles (Hemptinne et al. 1992; Kindlmann and Dixon 1993; Dixon 1997, 2000). The hypothesis states that the optimal reproductive strategy for a female lady beetle is to lay her eggs early in the development of a local aphid population (i.e., before aphid numbers peak), such that her offspring can complete development before aphid numbers decline severely and immature lady beetles become highly vulnerable to starvation, cannibalism and intraguild predation. Other field studies have also supported the egg window hypothesis (Hemptinne et al. 1992; Dixon 2000; Osawa 2000). In other cases, however, egg production of lady beetle populations was reported to peak simultaneously with aphid abundance (Wright and Laing 1980; Agarwala and Bardhanrov 1999).

Reproductive comparison between an invasive and a native lady beetle

The field data indicate that the invasive *C. septempunctata* has greater success in reproducing in the alfalfa habitat than does the native *C. transversoguttata*. Females of *C. septempunctata* produced eggs earlier in the season and a greater percentage of females were reproductively active than for the native species. In addition, gravid females of *C. septempunctata* produced more eggs than did gravid females of *C. transversoguttata*. Invasive *C. septempunctata* also more nearly reached their potential egg production as measured in the laboratory. Most of the larvae and pupae found in fields were individuals

of *C. septempunctata*, which suggests further that the invasive species was particularly successful in reproducing in this habitat.

Alfalfa is distinctive among valley habitats in the arid Intermountain West of North America. Following the introduction of this crop to the region in the mid nineteenth century, alfalfa fields have provided a very favorable, mesic environment for aphidophagous lady beetles (Evans 2000). The field data presented here indicate that alfalfa is a suitable habitat for reproduction by *C. septempunctata* in particular, and that this invasive species more than native species such as *C. transversoguttata* flourishes in this habitat. This in turn supports the hypothesis that alfalfa might serve as a source habitat for *C. septempunctata*. The large numbers of this invasive species may disperse widely from this source habitat as adults among many habitats, and that now dominate the lady beetle fauna of this geographic region.

Factors promoting reproductive success of C. septempunctata

Why is the invasive *C. septempunctata* able to reproduce early and obtain high fecundity in alfalfa fields in comparison to the native *C. transversoguttata*? The two species may differ in their foraging behavior, with important consequences in particular in spring when pea aphids occur in low numbers. Field data demonstrate that throughout the spring, females of *C. septempunctata* in alfalfa fields were more successful in achieving and maintaining high body weights as well as high rates of egg production (relative to maxima as established in the laboratory) than were females of *C. transversoguttata*. Such success may reflect foraging not only for preferred aphids, but also for a variety of other prey that aphidophagous lady beetles are known to consume

(Hagen 1987; Pemberton and Vandenberg 1993; Hodek and Honek 1996). In alfalfa fields of northern Utah, for example, the larvae of alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), are very abundant every spring and can serve as alternative prey when pea aphid populations are low (e.g., Webster 1911; Essig and Michelbacher 1933; Yakhontov 1934; Evans and England 1996; Richards and Evans 1998). Evans (2004) found from long-term censuses and field experiments that adults of *C. septempunctata* persist in alfalfa fields with low densities of aphids even as adults of native lady beetles emigrate to other habitats. Perhaps adults of *C. septempunctata* forage especially effectively for alternative prey such as alfalfa weevil larvae, and thereby maintain themselves well even at low aphid densities. In addition, invasive *C. septempunctata* may have greater ability to find pea aphids efficiently compared with native *C. transversoguttata* (L.N. Davidson, personal communication). Greater foraging ability may enable females of *C. septempunctata* to succeed more than *C. transversoguttata* in reproducing in alfalfa even in the presence of only low numbers of aphids (e.g., Evans 2004).

Differences in life-cycle phenology may also explain the earlier and greater reproductive activity of *C. septempunctata* versus *C. transversoguttata*. Lady beetles survive unfavorable periods by entering imaginal (including reproductive) diapause, which can be either obligatory (e.g., as in univoltine species) or facultative (as in species that potentially are multivoltine). Central and western European populations of *C. septempunctata* are strongly univoltine, although a few individuals in these populations are potentially multivoltine (Hodek and Honek 1996; also see Hodek and Michaud 2008). Many females of *C. septempunctata* enter reproductive diapause in summer (semi-estivo-

hibernation), even in the presence of excess prey (Hagen 1962; Hodek and Honek 1996). As seen also in the present study, Obrycki and Tauber (1981) and Phoofolo et al. (1995) noted a similar strong tendency to be univoltine with a high rate of reproductive diapause in *C. septempunctata* populations now established in North America. The greenhouse rearing study presented here indicates that the invasive *C. septempunctata* may have only a weakly expressed partial second generation each year, whereas the native *C. transversoguttata* appears to be much more fully bivoltine (with a further tendency for a partial third generation). These life-cycle differences may have physiological consequences for individuals of the two species that enter overwintering diapause each fall, as the exotic adults may have a longer period in the summer and fall to prepare for winter and the following spring. In particular, individuals of *C. septempunctata* may be able to accumulate more fat reserves before entering diapause, with one consequence that they emerge the following spring with relatively high fat reserves from which to initiate reproduction. Furthermore, females of the native *C. transversoguttata* that emerge in spring with low fat reserves appear unable to catch up with *C. septempunctata* females in this regard (e.g., as reflected in their lower body weights in the field throughout the spring, relative to the maxima of which they were capable). Such inability may result in part because, as observed in visual censuses in the present study, females of *C. transversoguttata* tend to arrive relatively later in alfalfa fields in the spring than do females of *C. septempunctata*.

Implications of the invasion of C. septempunctata for native lady beetles

Global biodiversity and the functioning of current ecological communities are threatened by many species invasions related to human activities and the consequent global homogenization of the biosphere (e.g., Lodge 1993; Mack et al. 2000). Classical biological control used instead of or in addition to chemical pesticides in pest management may pose risk through adverse non-target effects of exotic natural enemy introductions on native species in local communities (e.g., Simberloff and Stiling 1996; Thomas and Willis 1998). The highly successful invasion of North America in recent decades by introduced lady beetles, particularly *C. septempunctata* and *Harmonia axyridis* Pallas, has led to widespread concern about their effects on native lady beetles (e.g., Louda et al. 2003). In alfalfa fields of the Intermountain West, populations of *C. septempunctata* have increased greatly, while at the same time populations of *C. transversoguttata* and other native lady beetles have declined (Evans 2000, 2004). Similar patterns in other parts of North America (e.g., Elliott et al. 1996; Turnock et al. 2003; see also Brown and Miller 1998; Brown 2003) have stimulated great interest in potential causes of such species replacement.

Intraguild predation (IGP) is hypothesized as one mechanism for the successful establishment of *C. septempunctata* (Obrycki et al. 1998a; Kajita et al. 2000, 2006; Snyder et al. 2004; Yasuda et al. 2004). IGP by *C. septempunctata* adults on *C. transversoguttata* eggs or larvae could have contributed to the observed greater reproductive success of *C. septempunctata* in alfalfa fields. This was not addressed in the present study, however, and in general the importance of such IGP in accounting for

replacement of native lady beetles by *C. septempunctata* has yet to be demonstrated in the field (e.g., Obrycki et al. 1998b).

Exploitative competition may also be important in accounting for the displacement of native lady beetles by *C. septempunctata* in alfalfa fields studied here. Aphid populations in alfalfa during the present studies were low in comparison to densities recorded prior to the introduction of *C. septempunctata* (Evans 2004). In reaching high numbers while exploiting alfalfa as a source habitat, *C. septempunctata* may now be limiting the population growth of pea aphids. Consequently, native lady beetles may have abandoned alfalfa fields to forage elsewhere. As indicated by the results of the present study, females of the native *C. transversoguttata* achieve relatively little reproductive success while foraging in alfalfa fields with current, low aphid populations.

Study of reproductive biology provides essential information for understanding fundamental aspects of living organisms. The relationship between rates of successful invasion and reproduction has been reported in other insect taxa including introduced natural enemies for pest management (Crawley 1986), and in plants (Rejmanek and Richardson 1996). Additional attributes, such as excellent dispersal ability (Smith et al. 1999), physiological capacity to tolerate a broad spectrum of environmental conditions (e.g., Baker 1974; Sakai et al. 2000), and rapid adaptation in response to intense natural selection (Smith et al. 1999) may also contribute to successful invasions. The results of the present study provide insight as to the importance of reproduction in particular, in promoting successful invasion of *C. septempunctata* and its displacement of lady beetles in alfalfa fields of western North America.

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Table 3-1 Periods during which adult lady beetles and aphids were sampled in alfalfa fields in 2004 and 2005, with periods identified both by numbered sampling weeks and by dates.

Reproductive Period	2004	2005
Early (pre-week)	1–4 (April 19–May 16)	4–6 (May 9–May 30)
Peak		
Early peak	5 (May 17–24)	7 (May 31–June 6)
Mid peak	6–7 (May 25–June 7)	8–9 (June 7–20)
Late peak	8 (June 8–14)	10 (June 21–27)
Late (post-peak)	9–13 (June 15–July 19)	11–14 (June 28–July 25)

Table 3-2 Minimum and maximum air temperature in Logan, UT during April to June in 2004 and 2005. Data were from the Utah Climate Center.

	2004		2005	
	<i>Min</i>	<i>Max</i>	<i>Min</i>	<i>Max</i>
<i>April</i>	38.9 °F ± 0.8	60.3 °F ± 1.5	35.3 °F ± 0.9	52.6 °F ± 1.6
<i>May</i>	44.4 °F ± 1.1	66.9 °F ± 1.7	43.9 °F ± 0.9	66.4 °F ± 1.6
<i>June</i>	52.3 °F ± 0.8	77.8 °F ± 1.2	48.8 °F ± 1.5	73.6 °F ± 2.1

Table 3-3 Results of three-way ANOVA (species, year and period) for wet weight of females of *Coccinella septempunctata* and *C. transversoguttata* collected from alfalfa fields during early and peak periods in 2004 and 2005.

	DF	F	P
Species	1, 2062	270.76	< 0.0001
Year	1, 2062	30.62	< 0.0001
Species * Year	1, 2062	20.81	< 0.0001
Period	1, 2062	220.91	< 0.0001
Species * Period	1, 2062	26.09	< 0.0001
Year * Period	1, 2062	26.64	< 0.0001
Species * Year * Period	1, 2062	4.92	0.0266

Table 3-4 Numbers of individuals collected in sweep-net sampling for lady beetle larvae, and visual census (60 person-minutes) for pupae in 2004.

		<i>Animal Science</i>				<i>Caine Dairy</i>			
		<i>Larvae</i>		<i>Pupae</i>		<i>Larvae</i>		<i>Pupae</i>	
<i>Week</i>	<i>Date</i>	<i>C7^a</i>	<i>Others</i>	<i>C7</i>	<i>Others</i>	<i>C7</i>	<i>Others</i>	<i>C7</i>	<i>Others</i>
Week 8	11-Jun	–	–	–	–	–	–	6	–
Week 9	15-Jun	1	1(Hq ^b)	14	–	–	–	–	–
	16-Jun	15	1(Hs ^c)	13	1(Hc)	2	–	2	–
	17-Jun	3	–	20	1(Hc)	–	–	1	–
	18-Jun	12	1(Hq)	2	–	–	–	1	–
Week 10	25-Jun	10	1(Hq)	9	–	5	1(Hc)	4	–
	28-Jun	2	–	12	–	–	–	10	–
Week 11	29-Jun	3	2(Hc ^d)	15	–	1	–	–	–
	1-Jul	1	–	2	–	–	–	–	–
Week 12	9-Jul	–	–	–	–	2	–	1	–
	12-Jul	–	–	–	–	–	–	1	–
Total		47	6	87	2	10	1	26	0
%		88.7	11.3	97.8	2.2	90.9	9.1	100	0

C7^a represents *Coccinella septempunctata*, Hq^b represents *Hippodamia quinquesignata*, Hs^c represents *H. sinuata*, and Hc^d represents *H. convergens*.

Table 3-5 Percentages of females of successive summer generations of *Coccinella septempunctata* and *C. transversoguttata* that initiated reproduction (i.e., laid eggs versus entered reproductive diapause) when lady beetles were held and reared in the greenhouse (and thereafter in the laboratory under natural daylight beginning in mid-September).

<i>Generation</i>	<i>Date of adult emergence</i>	<i>C. septempunctata</i>	<i>C. transversoguttata</i>
1	July 12	45.0% (18 active / 40 total)	91.7% (27 active / 30 total)
2	August 15	7.7% (2 active / 26 total)	14.3% (2 active / 14 total)
3	September 30	5.0% (1 active / 20 total)	4.8% (1 active / 21 total)

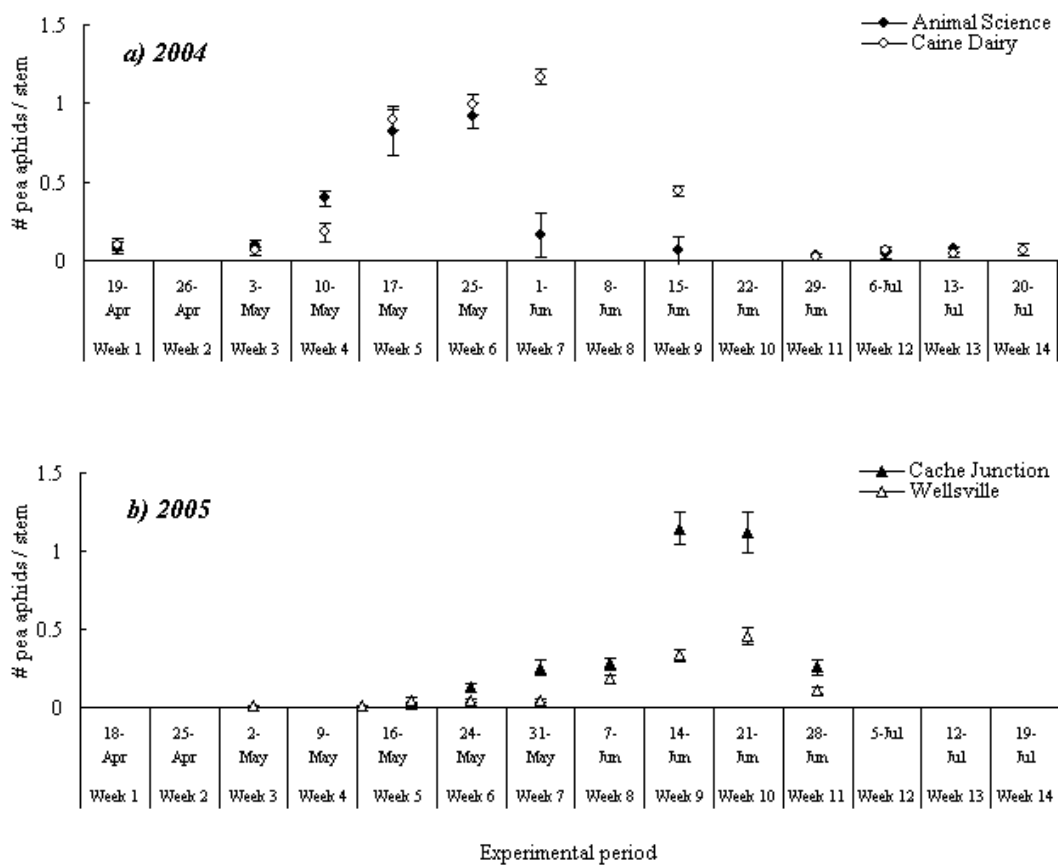


Fig. 3-1 The mean number of pea aphids per stem (\pm SE) that occurred in alfalfa fields sampled in (a) 2004 (Animal Science and Caine Dairy Farms) and (b) 2005 (Cache Junction and Wellsville Farms).

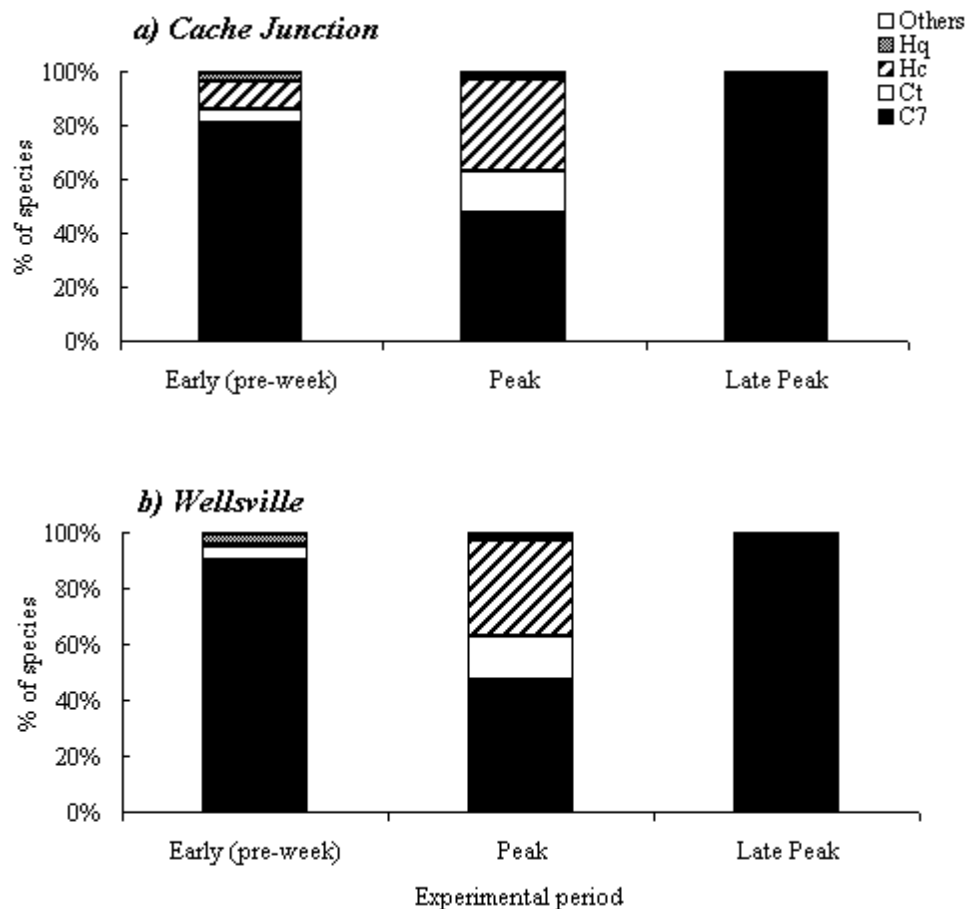


Fig. 3-2 Relative abundances of adult lady beetles, *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), *Hippodamia convergens* (Hc), *H. quinquesignata* (Hq), and *H. sinuata* (Hs), in different periods of 2005 in (a) Cache Junction and (b) Wellsville Farms, as recorded during 60 person-minute visual censuses.

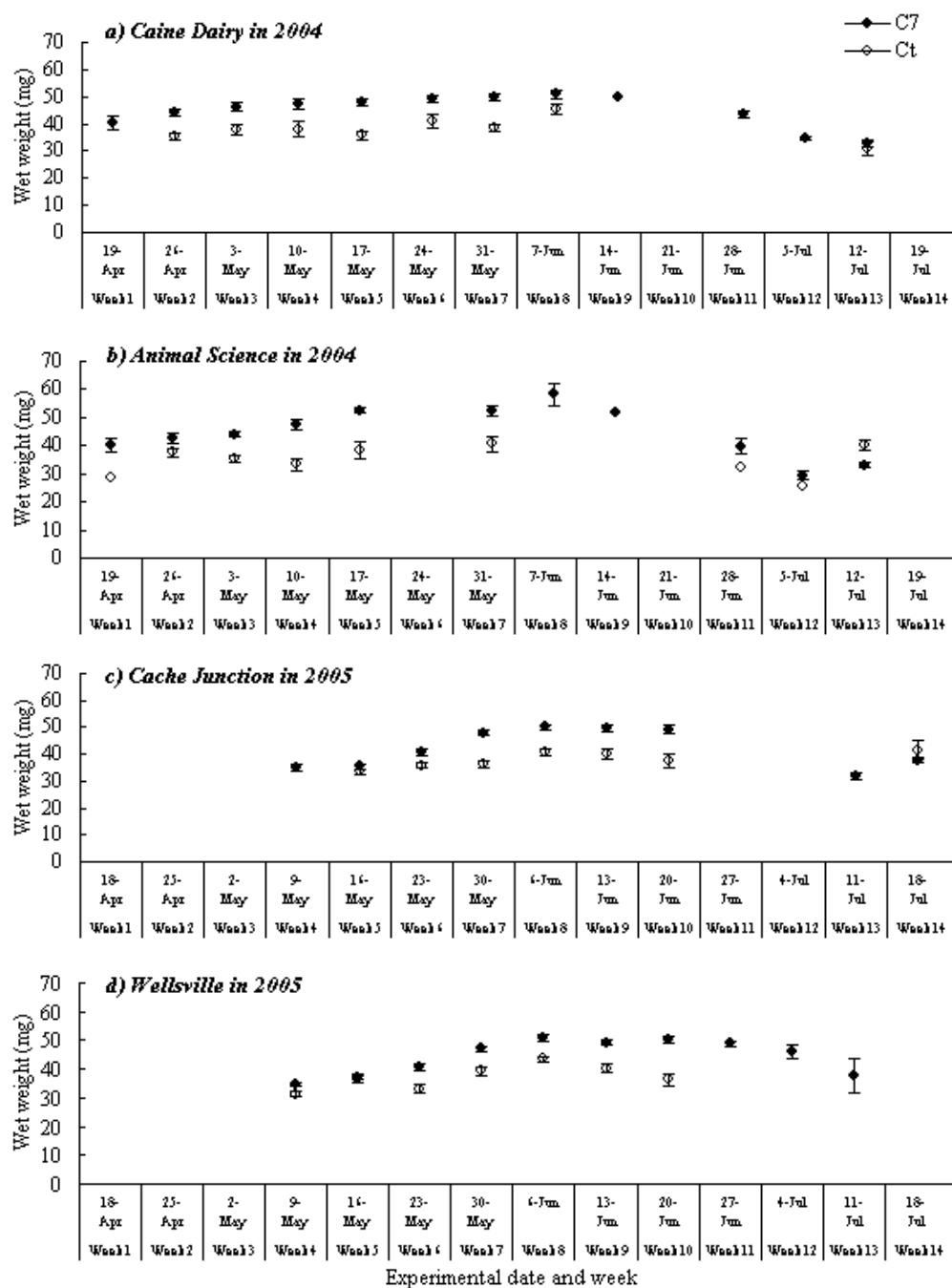


Fig. 3-3 Mean wet weights (\pm SE) of females of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) collected in different weeks in alfalfa fields at (a) Caine Dairy and (b) Animal Science Farms in 2004 and at (c) Cache Junction and (d) Wellsville Farms in 2005.

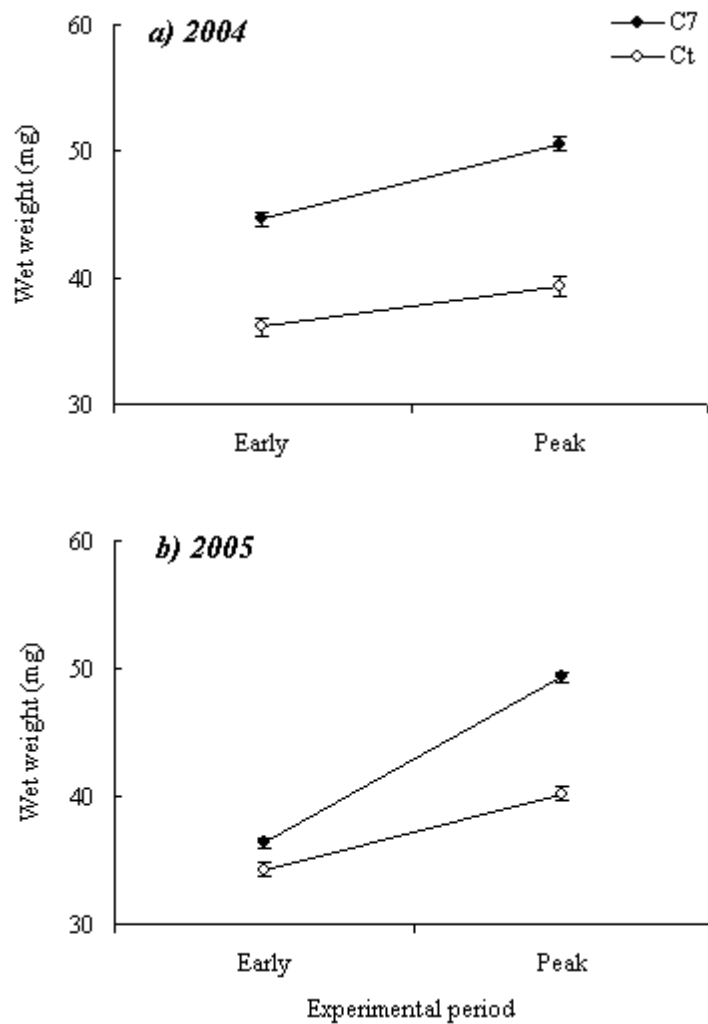


Fig. 3-4 Changes in mean wet weight (\pm SE) of females of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) collected in alfalfa fields between early and peak periods in (a) 2004 and (b) 2005.

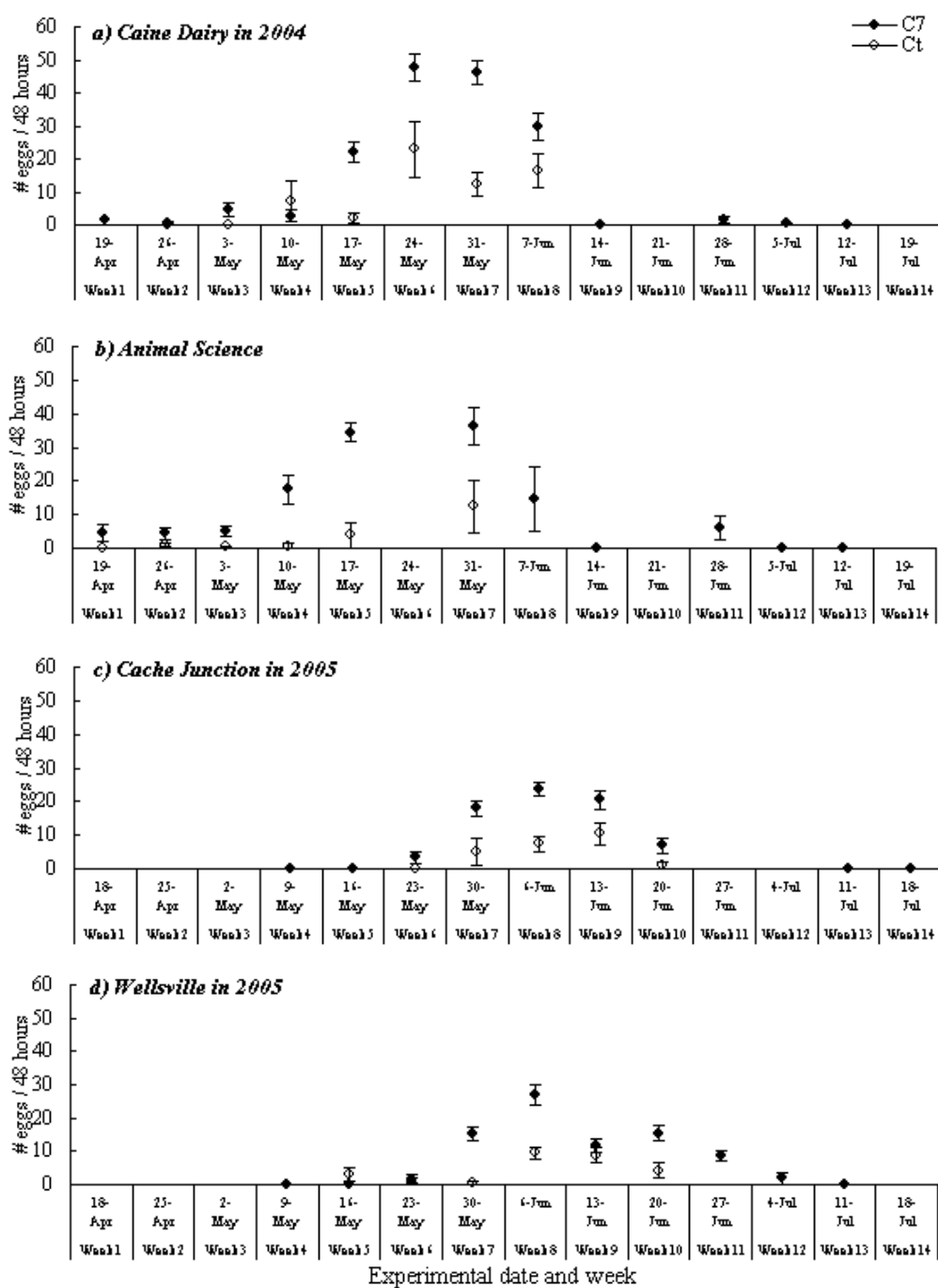


Fig. 3-5 The mean number of eggs (\pm SE) laid by females of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) over 48 hours after they were collected from alfalfa fields at (a) Caine Dairy and (b) Animal Science Farms in 2004, and at (c) Cache Junction and (d) Wellsville Farms in 2005.

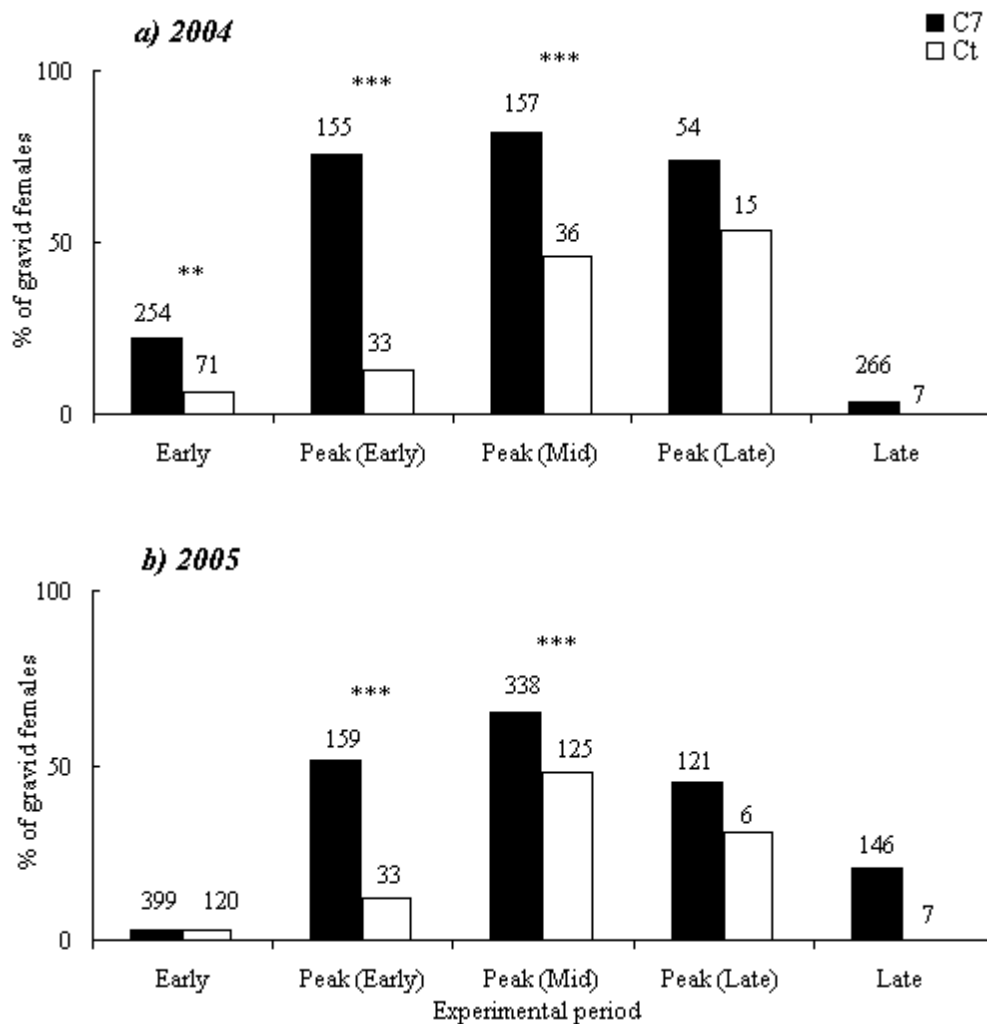


Fig. 3-6 The percentage of field collected females of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) that were gravid (i.e., that laid eggs over a 48 hour holding period) in (a) 2004 and (b) 2005. ** represents $P < 0.01$, and *** represents $P < 0.001$ by Monte Carlo Estimate ($n = 10,000$) for the exact test. $P = 0.73$ during early in 2005, $P = 0.20$ and $P = 0.25$ during peak (Late) in 2004 and 2005, respectively. $P = 1.0$ and $P = 0.59$ during late in 2004 and 2005, respectively. Number above the bars represents the total number of females collected in alfalfa fields (2 fields were combined) for each period in 2004 and 2005.

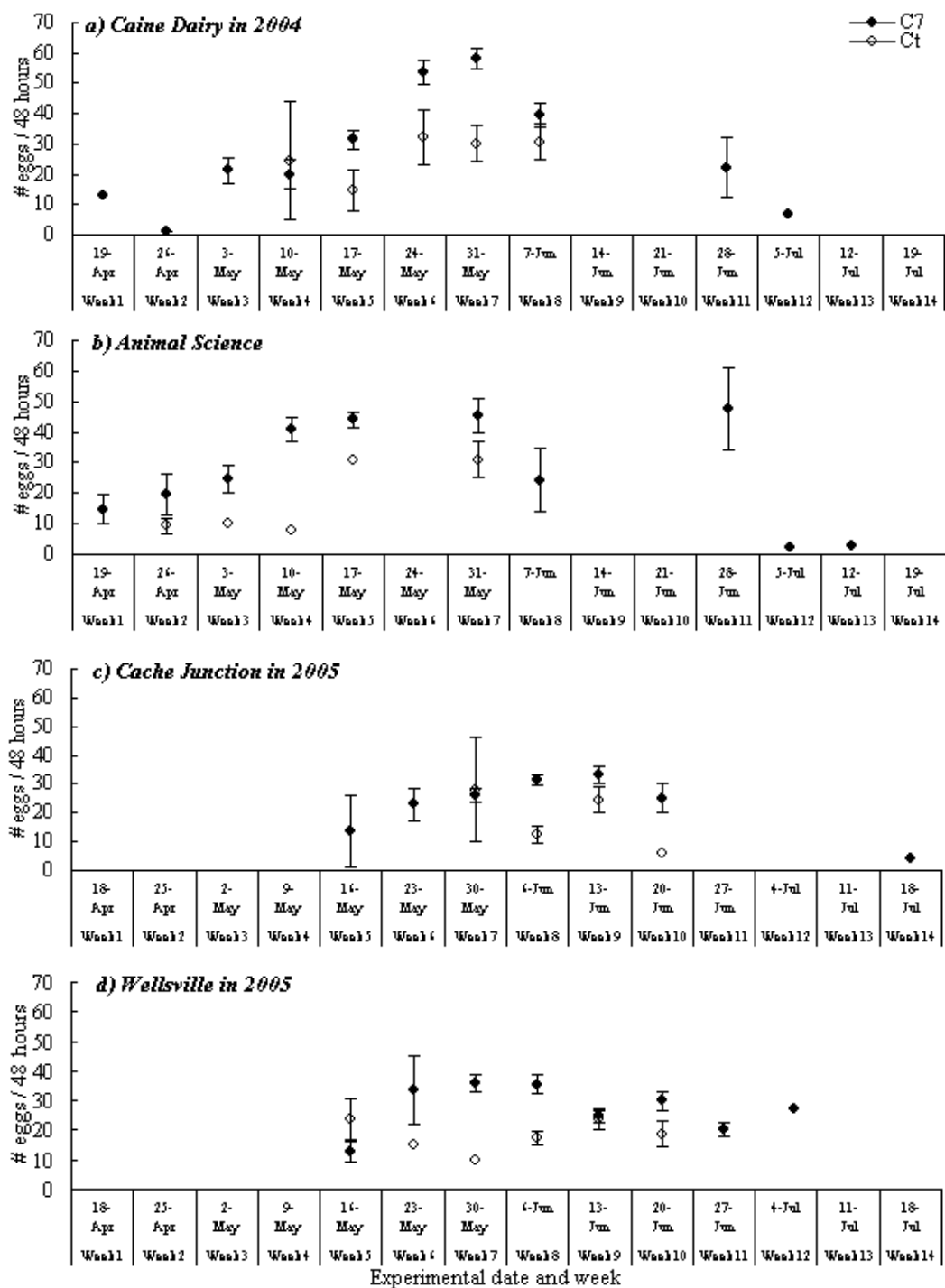


Fig. 3-7 The mean number of eggs (\pm SE) laid by gravid females of *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) over 48 hours after they were collected from alfalfa fields at (a) Caine Dairy and (b) Animal Science Farms in 2004 and at (c) Cache Junction and (d) Wellsville Farms in 2005.

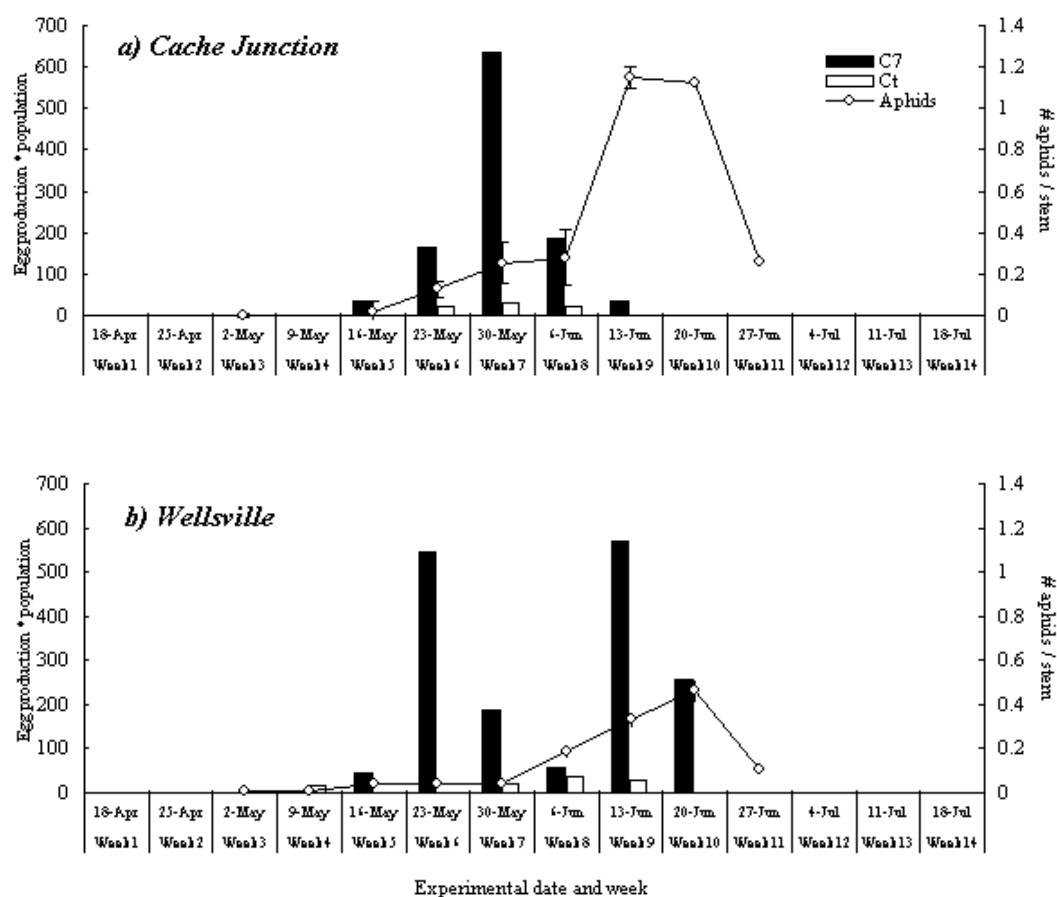


Fig. 3-8 Estimates of population-level relative egg production (determined as the mean number of eggs laid by a female [for all females combined] during a given week x the abundance of females that week as determined from visual censusing) for *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) and weekly mean aphid abundance in alfalfa fields (\pm SE) at (a) Cache Junction and (b) Wellsville Farms in 2005.

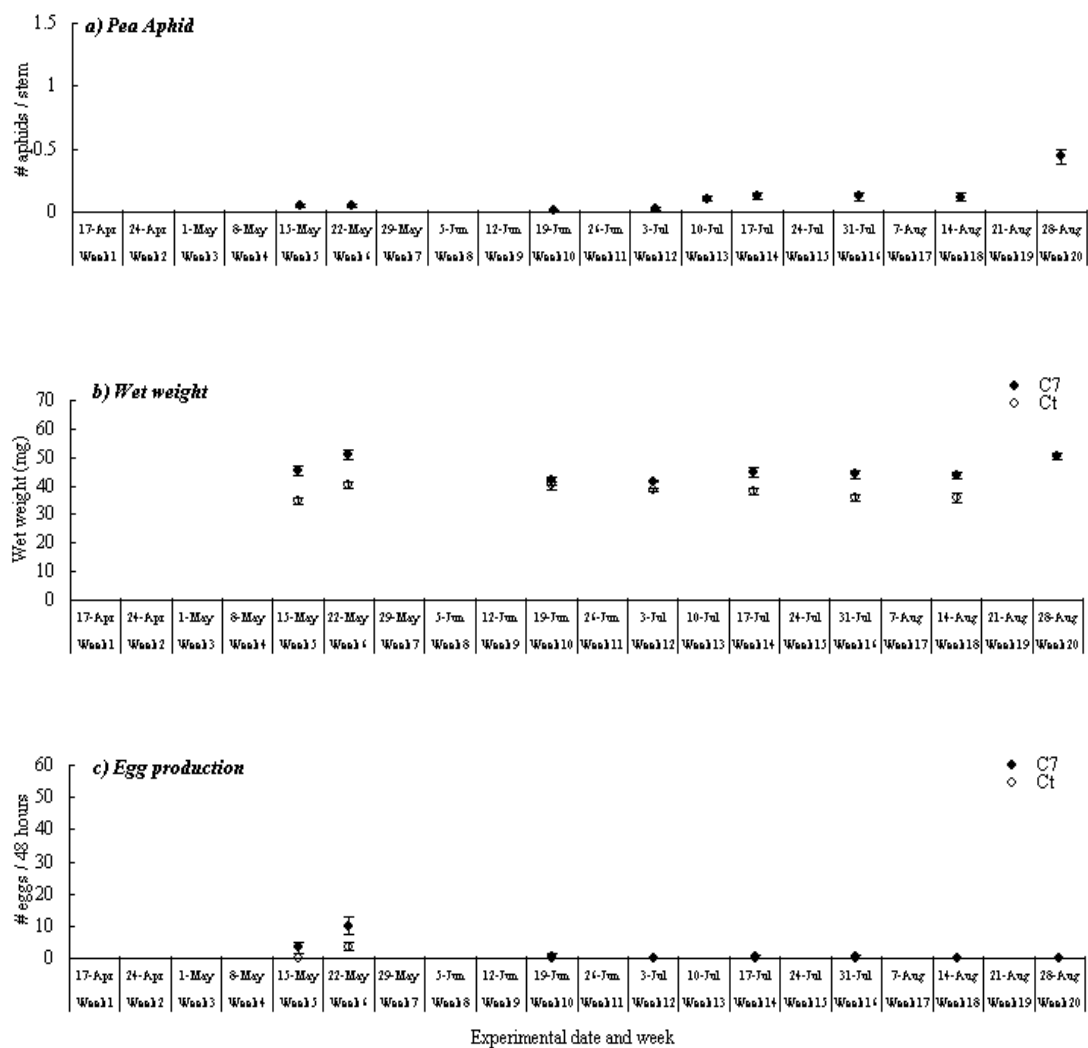


Fig. 3-9 Mean (\pm SE) (a) number of pea aphids, (b) wet weights, and (c) number of eggs over 48 hours for females *Coccinella septempunctata* (C7) and *C. transversoguttata* (Ct) in 2006 from an alfalfa field at the Wellsville Farm.

CHAPTER 4

COMPARATIVE REPRODUCTION OF TWO LADY BEETLES AT LOW
VERSUS HIGH PREY DENSITY

Abstract Prey populations usually vary greatly in size both spatially and temporally. Therefore, it is important to examine how predators such as aphidophagous lady beetles adjust constantly to such dynamic food environments to ensure their survival and the successful development of their progeny. Laboratory experiments were conducted to compare the reproductive responses of females of two species of lady beetles, *Coccinella septempunctata* and *C. transversoguttata*, to varying availability of prey. *C. septempunctata* is an introduced species that has been displacing the native species *C. transversoguttata* in alfalfa fields of northern Utah. To evaluate the potential role of reproductive tactics in accounting for this species replacement in this habitat, rates of reproduction of field-collected, overwintered females of the two species were measured in the lab in two experiments in which they were provided with varying numbers of pea aphids. When lady beetles were placed immediately upon experimental diets after being collected from the field (first experiment), females of *C. septempunctata* produced more but individually smaller eggs than did females of the similarly sized *C. transversoguttata*. Overall, however, the results of both experiments indicated that females of *C. septempunctata* and *C. transversoguttata* respond similarly in the numbers of eggs that they lay when they consume pea aphids in varying amounts. In particular, the experiments provided no evidence that females of *C. septempunctata* convert pea aphids into eggs at a relatively higher rate than females of *C. transversoguttata* do when prey availability is limited. On the other hand, *C. septempunctata* females had greater ability

than *C. transversoguttata* females to maintain their body weights, even as they were producing eggs at low rates when aphids were provided in limited numbers. This suggests that low aphid availability is less stressful for reproducing females of *C. septempunctata*, perhaps because females of *C. septempunctata* have more ability physiologically to assimilate pea aphid nutrients (many of which are used for self-maintenance) at low aphid availability, compared with reproducing females of *C. transversoguttata*.

Furthermore, overwintered females of *C. septempunctata* may arrive in alfalfa fields with greater nutrient reserves, thereby also enabling them to reproduce more quickly thereafter than females of *C. transversoguttata*. Further studies are needed to determine if females of *C. septempunctata* are in better physiological condition than are females of *C. transversoguttata* in spring alfalfa fields. Such studies, combined with the results of the experiments presented here, may provide insight into why the introduced lady beetle has become much more abundant than its native congener in this habitat.

Introduction

Life history theory explores how organisms vary in their specific strategies for reproductive success in varying environments. For predatory insects, prey availability may differ widely among habitats. This may affect rates of prey consumption and egg production of predators. In most insect predators, rates of egg production increase with prey availability (Dixon 1959; Ives 1981; Mills 1981; Coll and Ridgeway 1995).

However, the patterns of change in egg production with prey availability may differ among predators, even in closely related species (Landahl and Root 1969; Peschken 1972; Nakashima and Hirose 1999). These differences may reflect different reproductive

strategies.

Not only egg production, but also progeny size and fitness (as reflected in offspring growth and survival), may vary in different environments as the result of maternal responses. Maternal responses are influenced by factors such as paternal physiological conditions, maternal diet and density, food availability, seasonal variation and age of females (reviewed by Fox and Czesak 2000). For example, maternal diet can affect egg size and egg fertility. In general, it is expected that as food stress increases and reduces the chances of offspring survival, females should invest more in each offspring (Clutton-Brock 1991; Roff 2002).

In predatory lady beetles (Coleoptera, Coccinellidae), it is well documented that food availability affects the rate of egg production such that few eggs are laid when food availability is low (Dixon 2000). Aphidophagous lady beetles require aphids as food to maintain themselves as adults and to stimulate reproductive activity (e.g., Evans and Dixon 1986; Richards and Evans 1998). Aphid populations are ephemeral and often display boom-bust dynamics in the field. Therefore, lady beetle females must forage widely and adjust to a constantly changing prey environment to ensure their survival and to maximize also the chances of survival of their progeny.

In this study, the case of *Coccinella septempunctata* L. and its recent invasion of the intermountain west in the United States (e.g., Evans 2000, 2004) are considered. Since its arrival and initial establishment in the early 1990s, *C. septempunctata* has consistently maintained high populations in alfalfa fields while at the same time, the densities of native lady beetles have declined (Evans 2000, 2004). One adversely affected species is the native congeneric species, *C. transversoguttata* Brown (Evans 2004). In a

related study (Chapter 2), it was found that under laboratory conditions, *C. septempunctata* females produce more and individually smaller eggs than *C. transversoguttata* females do when pea aphids (*Acyrtosiphon pisum*) are provided in excess. However, it is unknown how *C. septempunctata* and *C. transversoguttata* compare in their reproductive responses to limited availability of prey. A field study (Chapter 3) suggested that *C. septempunctata* may maintain a higher rate of reproduction than *C. transversoguttata* even when consumption rates of pea aphids are low. Here laboratory experiments were performed to test directly whether *C. septempunctata* females have higher rates of reproduction than females of *C. transversoguttata* do when aphids are provided as prey in limited numbers. Additionally, the experiments tested whether *C. septempunctata* adjusted their reproductive effort in response to short-term changes in prey availability more quickly in comparison with females of *C. transversoguttata*. To address these questions, two laboratory experiments were conducted. In 2005, an experiment was conducted to compare the rates of reproduction between field-collected adults of *C. septempunctata* and *C. transversoguttata* when they were transported to the lab and immediately were provided with limited quantities of prey. In 2006, field-collected females of *C. septempunctata* and *C. transversoguttata* were first provided with prey in excess for two weeks, before they were then switched to feeding regimes of different prey availabilities (high, low or intermittent [high numbers or no prey provided on alternating days]) as well as a control treatment of continuing provision of excess prey.

Materials and Methods

Reproductive comparison between high versus low prey density (the first experiment)

In the first experiment, rates of reproduction were compared between overwintered females of *Coccinella septempunctata* and *C. transversoguttata* that were taken directly from the field in early spring, and were provided with either high or low numbers of aphids in the laboratory thereafter. Adults of the two species of lady beetles were collected in late April to early May 2005 in nearly equal numbers from each of two alfalfa fields near Logan, Utah. These adults had recently arrived in the alfalfa fields from overwintering sites, and were in the early stages of becoming reproductively active (see Chapter 3). In the lab, females and males were immediately paired and provided for the next thirty days with one of two (high or low) levels of food daily: either 5 or 10 adult pea aphids per pair. Each pair was placed in a Petri dish (5.7cm in diameter and 1.5cm in height) in an incubator at 22°C, 16L:8D, for the experimental period of 30 days. On each day of the experiment, each pair of lady beetles was transferred to a new Petri dish provisioned with a fresh set of pea aphids [reared on fava bean, *Vicia faba* L. (Fabaceae), in the greenhouse]. One drop of water was added on the underside of the lid to add humidity within the Petri dish. A strip of folded filter paper was also put in each Petri dish, both to create more oviposition sites for females and to minimize egg cannibalism by males and their disturbance of ovipositing females. Females that died during experiments were not replaced. Dead males were replaced, however, so that females remained paired with a live male throughout the experiment. The final sample sizes of females that survived the full 30 days were 24 and 19 *C. septempunctata* females for low

and high prey densities, respectively, and 19 and 18 *C. transversoguttata* females for low and high prey levels, respectively.

Wet weights of females were measured every other day throughout the experiment, beginning on the day of collection. The number of eggs produced by each female was recorded each day (at the time that pairs were transferred to new Petri dishes each morning). Egg cannibalism occurred only rarely. When egg cannibalism occurred, the residues of eggs could be identified, and cannibalized eggs were added into counts of daily egg production.

On each of 3 days during the experiment (Day 7, 17, and 27), the length and width of 5 randomly selected eggs per female were measured using a SZX Olympus stereomicroscope at 30 x magnification, for those females that laid eggs. Egg volume (mm^3) was calculated from these measurements, following Phoofolo et al. (1995) and Richards and Evans (1998), by using the formula for a prolate spheroid, $(4/3)\pi ab^2$, where a is equal to half the egg length and b is equal to half the egg width. A mean egg volume was then calculated for each female over the entire 30-day period (i.e., a mean based on up to 15 eggs, although not all females laid eggs on each of the 3 days). The total volume of eggs laid during the 30-day experimental period was calculated from the total number of eggs produced multiplied by mean egg volume of each female over the entire 30-day period.

Egg viability was measured on every third day. One egg batch was taken from each female that laid eggs, and put in an incubator at 22°C, 16L:8D, for several days until eggs hatched. The emergence of hatchlings was checked every 4 to 6 h; to prevent sibling egg cannibalism, hatchlings were removed immediately. From these observations, the

overall percentage of eggs laid by each female that were fertile was calculated.

Females were frozen at the end of the experiment. Subsequently, the length of an elytron, and the widths of the pronotum, and the abdomen, of each female were measured. Because these three measurements are highly correlated with each other ($r=0.3\sim 0.8$, $P<0.05$, in all cases), elytron length was selected as a measure of overall body size.

The wet weights of *C. septempunctata* and *C. transversoguttata* females upon collection, and after 24 h on lab diets, were compared by paired t tests. Thereafter, the wet weights of females as measured daily during the 30-day experiment were compared between the two species and among two food levels using two-way repeated measures of analysis of variance (ANOVA). Repeated measures of ANOVA in Proc Mixed with Huynh-Feldt covariance structure was used for this analysis by using SAS 9.1 (SAS Institute 2002–2003).

The total numbers of eggs laid by females of *C. septempunctata* and *C. transversoguttata* during each 10-day period and the full 30-day experimental period were calculated. Two-way repeated measures of ANOVA were conducted to analyze these totals with square root transformation. Regression analyses between body size of females (elytron length in mm) and their total egg production for 30 days were also conducted.

Because egg volume and viability varied little over the course of the experiment, mean egg volume and viability for individual females were also calculated based on the full 30-day period. Egg volume and viability were analyzed by two-way ANOVA. Total volume of eggs laid for the full 30-day experimental period was calculated as mean egg

volume x total egg production over the full 30 days for individual females. Total volume of eggs was transformed by square root, and then analyzed by two-way ANOVA. All analyses were performed by using SAS 9.1 (SAS institute 2002–2003).

Comparative reproductive ability in response to varying prey density

(the second experiment)

In the second experiment, rates of reproduction were compared between overwintered females of *C. septempunctata* and *C. transversogutatta* that were collected in the field in early spring, maintained at first in the laboratory on *ad libitum* diets of aphids, and thereafter provided with varying levels of food. Adults of the two species of lady beetles were collected in late April to early May 2006 as described above for the first experiment (completed in 2005). In the lab, they were immediately paired, held in Petri dishes as described above, and offered excess numbers of aphids for the next 14 days. During this initial period, daily numbers of eggs laid by each female were recorded. Beginning on the fifteenth day, twelve pairs of both species were assigned to one of four levels of food (pea aphids) for the next 10 days (i.e., the first experimental period: Days 15 to 24): (1) *ad libitum* (a continuation of excess numbers of aphids provided each day), (2) high (10 aphids per pair per day), (3) low (5 aphids per pair per day), or (4) intermittent (10 aphids per pair provided every other day, with no prey provided on alternate days). To encourage greater egg production as the experiment continued, food levels were adjusted upward for a second 10-day period (Days 25 to 34) such that pairs received 18 or 9 aphids every day, or 18 aphids every other day, for high, low, and intermittent levels of food, respectively. During the final 10-day period (Days 35 to 44),

food levels were again increased, to 20 or 10 aphids every day, or 20 aphids every other day, for high, low, and intermittent levels of food.

Procedures followed during the experiment in general were the same as those described above. The females' wet weights were measured on the fifth and tenth day of each period (i.e., every 5 days). Egg production was quantified daily, again with the few cannibalized eggs added in. Total numbers of eggs laid during each of the three periods was calculated, with the totals for the first period based only on the final eight days (the first two days of this period were omitted, as egg production likely was determined on these days primarily by the excess numbers of aphids that all females consumed prior to the initiation of the experiment; Richards and Evans 1998). To calculate mean egg volume, the length and width of 5 randomly selected eggs from one egg batch produced by each female were measured daily from the third day (Day 18 from the day of collection in the field) on throughout the experiment. Thereafter, the mean egg volume for each individual during each experiment period was calculated. Total volume of eggs laid in each experimental period was calculated from mean egg volume multiplied by the number of eggs laid during each experimental period. Egg viability was determined for all eggs laid on every third day. Females were frozen at the end of the experiment. Subsequently, the length of an elytron, the pronotum and the abdomen of each female were measured.

Hatchling survivorship was examined during Days 15 to 17, when pairs had been switched to treatments of different prey availabilities. 50 eggs produced by females on these days in each treatment (i.e., excess, high, low and intermittent) were selected from egg batches randomly, and then kept in a Petri-dish (5.7cm in diameter and 1.5 cm in

height) individually with wet filter paper in an incubator (22°C, 16L:8D). Each egg in a Petri dish was observed every 3 h for hatching. After hatchlings emerged, their survivorship was measured every 3 h until they died.

Wet weights of females of *C. septempunctata* and *C. transversoguttata* on Days 1 and 14 (i.e., at the beginning and end of the period in which all pairs were provided with aphids *ad libitum*) were compared by paired t tests. Thereafter, wet weights on Day 20 for the first period, Day 30 for the second period, and Day 40 for the third period, were compared between species and among the four food levels by two-way ANOVA.

Total egg production during the initial 14 days when aphids were provided *ad libitum* was analyzed by one-way ANOVA to compare between species. Thereafter, the number of eggs produced by females during each of the three experimental periods was analyzed by two-way ANOVA with square root transformation, to compare the effects of species, food levels and their interaction.

Mean egg volume from daily measurements was calculated for each female in each experimental period, and then analyzed for effects of species and food levels by two-way ANOVA. Total volume of eggs laid per experimental period was calculated for each female by multiplying mean egg volume times number of eggs laid for each experimental period. Data were transformed by square root, and then analyzed by two-way ANOVA.

Mean egg viability was calculated by the number of hatchlings divided by the egg production per egg batch per day for each female during each experimental period, and then analyzed by two-way ANOVA. Hatchling survivorship was also analyzed by two-

way ANOVA. All analyses were performed by using SAS 9.1 (SAS institute 2002–2003).

Results

Reproductive comparison between high versus low prey density

Pairs at both high and low food levels ate all or most of the aphids provided each day throughout the experiment. Upon transfer from field to lab conditions, females of both *Coccinella septempunctata* and *C. transversoguttata* immediately gained weight (Fig. 4-1; *C. septempunctata* $t_{42}=-13.19$, $P<0.0001$, *C. transversoguttata* $t_{36}=-8.26$, $P=0.001$). Thereafter, there were significant effects of species and food levels, and a significant interaction, on wet weights of females as measured every other day (two-way repeated measures of ANOVA from Day 1 to 30: effect of species $F_{1,1140}=149.90$, $P<0.0001$, effect of food level $F_{1,1140}=133.99$, $P<0.0001$, interaction of species x food level $F_{1,1140}=24.35$, $P<0.0001$). There was also a significant effect of time ($F_{14,1140}=5.50$, $P<0.0001$) but no significant interactions with time ($P>0.1$ for species x time, food level x time, and species x food level x time). The significant interaction of species with food level reflects that females of *C. septempunctata* provided with a low level of food maintained body weights more similar to body weights of females provided with a high level of food than did females of *C. transversoguttata* (Fig. 4-1).

Females of *C. septempunctata* produced more eggs than did females of *C. transversoguttata* (Fig. 4-2a,b; effect of species in two-way repeated measures of ANOVA: $F_{1,76}=19.74$, $P<0.0001$). Both species produced more eggs when provided with more prey (effect of food level: $F_{1,76}=33.15$, $P<0.0001$), and produced more eggs after

the first 10 days (effect of time [i.e., three 10-day periods]: $F_{2,152}=37.01$, $P<0.0001$).

Females of the two species responded similarly to the two food levels (interaction of species x food level: $F_{1,76}=0.32$, $P>0.57$), and no significant interactions of time with species or food level effects occurred. The total number of eggs laid by a female over the entire 30-day experimental period was positively related to her body size at high food level (i.e., when 10 aphids were provided to a pair each day), but not at low food level (i.e., when only 5 aphids were provided), for both *C. septempunctata* and *C. transversoguttata* (Fig. 4-2c,d).

Females of *C. transversoguttata* laid larger eggs than did females of *C. septempunctata* (Fig. 4-3a; two-way ANOVA, effect of species: $F_{1,72}=72.49$, $P<0.0001$). There was not a significant difference between food levels, and the interaction of species x food level was not significant, for egg volume (Fig. 4-3a; effect of food level: $F_{1,72}=2.64$, $P=0.11$; interaction of species x food level: $F_{1,72}=1.71$, $P=0.19$).

There was a significant difference between food levels in the total volume of eggs laid during the full 30-day experimental period ($F_{1,65}=109.91$, $P<0.0001$), and there was a marginally significant difference between species ($F_{1,65}=3.53$, $P=0.065$), but the interaction of species x food level was not significant (species x food level: $F_{1,65}=0.14$, $P=0.71$). Overall, *C. septempunctata* and *C. transversoguttata* females laid 2.80 times, and 3.03 times more total volume of eggs, respectively, at high versus low food level (Fig. 4-3b).

The percentages of eggs laid by females of *C. septempunctata* and *C. transversoguttata* that were viable did not differ between the two species (Fig. 4-3c; two-way ANOVA, effect of species: $F_{1,60}=0.16$, $P=0.69$). The two species responded

similarly to high versus low food levels in producing a lower percentage of eggs that were viable at the low food level (effect of food level: $F_{1,60}=30.4$, $P<0.0001$; interaction of species x food level: $F_{1,60}=1.99$, $P=0.16$).

Comparative reproductive ability in response to varying prey density

During the experiment, pairs maintained on high, low and intermittent food levels ate all or most of the aphids provided each day. Upon transfer from field to lab conditions, females of both *C. septempunctata* and *C. transversoguttata* gained weight over the next two weeks, with prey provided *ad libitum* (paired t test for females' wet weights on Days 1 and 14: *C. septempunctata* $t_{47}=-13.53$, $P<0.0001$, *C. transversoguttata* $t_{46}=-16.05$, $P<0.0001$). Thereafter, the wet weight of females differed significantly between species and among food levels during each of the three experimental periods (Fig. 4-4a,b and Table 4-1). Although significant interactions between effects of species and food level were not detected during the first and second periods (Table 4-1), a consistent pattern associated with food levels was apparent across the three periods only for *C. transversoguttata* females. Weights of females of *C. transversoguttata* in all three periods were substantially lower when these females were provided with a low level versus an *ad libitum* or high level of food, whereas weights of females provided with a high level of food every other day (i.e., intermittent food level) were intermediate between low and high or *ad libitum* food levels. Females of *C. septempunctata* showed a similar, although less pronounced, pattern during the first period, but differed in inconsistent fashion among the second and third periods in their relative weights when maintained at a given food level (Fig. 4-4).

Females of *C. septempunctata* laid significantly more eggs than did females of *C. transversoguttata* during the first 14 days, after being collected from the field and while provided with prey *ad libitum* (one-way ANOVA for the total number of eggs laid in 14 days: species $F_{1,87}=43.80$, $P<0.0001$; mean total egg production for *C. septempunctata*: 450.5 ± 21.9 , and for *C. transversoguttata*: 267.3 ± 15.9). In each of the three experimental periods thereafter, females of *C. septempunctata* and *C. transversoguttata* laid similar numbers of eggs at a given food level, and were similar in the different numbers of eggs that they laid among food levels (Fig. 4-4c,d and Table 4-1). Overall, the females laid most eggs when prey were provided *ad libitum*, large numbers of eggs when high numbers of prey were provided, and low numbers of eggs when low numbers of prey each day or high numbers of prey every other day (intermittent treatment) were provided (Fig. 4-4c,d). Females at high food levels more nearly matched the egg production of females provided prey *ad libitum* during the second and third versus the first period, reflecting the increase in numbers of aphids provided at the high level in the latter two periods. Females of *C. septempunctata* in particular appeared to produce more eggs when low numbers of prey were provided daily versus high numbers of prey every other day (Fig. 4-4c,d). However, there were not significant interactions of species with food levels during experimental periods (Table 4-1).

Egg volume differed significantly between species and among food levels during each of the three periods (Table 4-2). No significant interactions occurred between species and food level (Table 4-2). As in the first experiment, females of *C. transversoguttata* laid larger eggs than females of *C. septempunctata* (Fig 4-5a,b and Table 4-2). Egg volume for females that were given excess prey remained relatively

constant throughout the experimental period. Females of both species, in general, laid slightly smaller eggs during the first period when the amount of food was decreased from *ad libitum* to high, low, and intermittent levels. As the amount of food provided daily was increased from the first to the second period, and again for the third period, females of both species laid slightly larger eggs, and by the third period, there was not a significant difference among food levels in their effects on egg volume (Fig. 4-5a,b and Table 4-2).

The total volume of eggs laid per experimental period (egg volume x egg production) was calculated for each of the three experimental periods (Fig. 4-5c,d). A significant difference occurred in the total volume of eggs produced by *C. septempunctata* and *C. transversoguttata* during the first period, reflecting the production of relatively larger eggs by *C. transversoguttata*. However, significant differences did not occur between the two species in the total volume of eggs laid during the second and third periods (Table 4-2). Significant differences occurred among food levels for the two species in each of the three periods (Table 4-2). As with egg production, *C. septempunctata* and *C. transversoguttata* females produced more total volume of eggs when maintained on *ad libitum* and high food levels than on low and intermittent food levels. There was not a significant interaction between species and food levels for any of the three experimental periods (Table 4-2).

Egg viability differed significantly between species in each period (Fig. 4-5e,f and Table 4-2). Egg viability also differed significantly in the first period among food levels (Table 4-2). With numbers of prey increased for low, high, and intermittent food levels during the second and third periods, the effects of food level on egg viability were weakened (Table 4-2). In general, while more of the eggs laid by *C. septempunctata* vs.

C. transversoguttata females were viable, greater percentages of eggs laid by both species at *ad libitum* or high versus low food levels were viable (Fig. 4-5e,f). Viability levels were consistently high among the three periods for *C. septempunctata* eggs laid when food was provided at high levels every other day, but were low (even more so than for eggs laid at low food levels) for *C. transversoguttata* eggs. Variances were high, however, such that the interaction between effects of species and food level was not significant during the experimental periods.

Hatchling survivorship varied among eggs laid by females in different treatments on Days 15 to 17. There were significant differences in hatchling survival between the two species, and among the food level treatments (Fig. 4-6; two-way ANOVA: species $F_{1,329}=9.50$, $P=0.0022$, treatments $F_{3,329}=13.15$, $P<0.0001$). The hatchlings of *C. transversoguttata*, which lays larger eggs, survived significantly longer than those of *C. septempunctata*. The hatchlings of both species survived the longest when they came from eggs laid by females with excess prey availability. Successively shorter survival times were recorded for hatchlings from eggs laid by females with high, low and intermittent prey availabilities. There was no interaction effect between species and treatments (species x treatments: $F_{3,329}=0.89$, $P=0.45$).

Discussion

The laboratory experiments presented here were designed to compare how *Coccinella septempunctata* and *C. transversoguttata* respond reproductively to limited availability of prey. Such comparison might help us understand the relatively high rate of reproduction that *C. septempunctata* achieves in alfalfa fields (Chapter 3). For example,

the experiments addressed whether females of *C. septempunctata* produce relatively more eggs at low prey availability than do females of *C. transversoguttata*. The experiment in 2005 was conducted to compare the rates of reproduction of overwintered adults of *C. septempunctata* and *C. transversoguttata* taken directly from the field and provided immediately with limited quantities of prey. The experiment in 2006 was conducted to compare rates of reproduction after females of *C. septempunctata* and *C. transversoguttata* had become very active in laying eggs as the result of feeding on excess prey for an extended period. Once they had become very active reproductively, females of both species were switched to various prey availabilities (high, low, and intermittent availabilities), while other females continued to be provided with excess prey. In 2005, field collected females of *C. septempunctata* produced more eggs than females of *C. transversoguttata* throughout the experimental period. They also did so during the first 14 days in 2006, while all females received excess prey. Thereafter, however, reproductive rates of the two species became similar, as discussed below.

In 2005, because field-collected females of *C. septempunctata* and *C. transversoguttata* were immediately used for the experiment, their physiological condition as determined in the field was an important factor in their responses to experimental treatments. Throughout the experiment, females of *C. septempunctata*, weighed more, and produced a larger number of eggs of smaller individual size than did females of *C. transversoguttata*, when aphids were provided at both high and low availability. In general, however, females of the introduced and native species responded similarly to treatments. The increase in total volume of eggs produced when more versus fewer aphids were provided each day was very similar between the two species. When

relatively large numbers of aphids were supplied each day, body size was an important determinant of the rate of production for both species. However, when few aphids were supplied each day, females of each species laid similarly few eggs regardless of body size. Egg volume did not differ between low and high food availability; however, egg viability varied in both species in response to low versus high aphid availability. A difference was apparent in the response of the two species to treatment in that females of *C. septempunctata* maintained similar body weights in response to low versus high aphid availability, but females of *C. transversoguttata* weighed less when provided daily with fewer aphids.

In 2006, an effort was made at the outset to reduce physiological differences between overwintered females of the two lady beetle species that might be present at the time of field collection. Therefore, females of both *C. septempunctata* and *C. transversoguttata* were provided with aphids in excess for two weeks after collection, before the experiment was conducted. In general, females of the two species responded similarly to treatments during the experiment. Females laid more eggs as more prey were provided, and laid similarly low numbers of eggs (and total egg volume) when provided either with a low number of aphids daily, or with twice this number of aphids every other day (and no aphids on days in between; “intermittent” treatment). During the third experimental period, females of *C. septempunctata* were distinctive in responding to intermittent prey availability by producing relatively few eggs (the interaction of species and food levels was not significant, however). Both species produced slightly larger eggs as the number of aphids provided for a given food level was increased from the first to the second and then to the third period. Overall, egg viability levels were lower among

eggs produced when aphids were provided in low rather than high (or excess) numbers, and were lower at all prey levels for eggs laid by *C. transversoguttata* versus *C. septempunctata*. The difference in levels of viability between species was especially apparent for eggs laid when aphids were provided intermittently (every other day). Another difference between the two species can be noted in body weights maintained by females at low versus high prey availability. As in 2005, females of *C. septempunctata* generally maintained high body weights throughout the experiment regardless of the quantity of prey supplied daily. In contrast, again as in 2005, females of *C. transversoguttata* tended to maintain relatively low body weight when provided with low numbers of aphids each day.

Overall, the combined results of the two experiments indicate that females of *C. septempunctata* and *C. transversoguttata* respond similarly in the numbers of eggs that they lay when they consume differing numbers of pea aphids. In particular, the experiments provided no evidence that females of *C. septempunctata* convert pea aphids into eggs at a relatively higher rate than females of *C. transversoguttata* do when prey availability is limited. On the other hand, *C. septempunctata* females had greater ability to maintain their body weights even as they were producing eggs at low rates when aphids were provided in limited numbers. This suggests that low aphid availability may be less stressful for reproducing females of *C. septempunctata*, perhaps because these females (versus *C. transversoguttata* females) have greater physiological efficiencies of conversion in assimilating nutrients from pea aphids at low availability (which they use more for self-maintenance [e.g., fat accumulation] rather than increased egg production).

The experiments revealed interesting differences in sizes of eggs laid by females

of both lady beetle species in response to treatment in 2006. Egg size is an important life history variable that is influenced by food availabilities through maternal physiological condition in insects (e.g., Wiklund and Persson 1983; Solbreck et al. 1989), including predatory insects (Gilbert 1990; Stewart et al. 1991). Egg size influences offspring survival in insects (Solbreck et al. 1989; Wallin et al. 1992), and the developmental time (Stewart et al. 1991; Obrycki and Tauber 1981).

In the present experiments, females of *C. transversoguttata* consistently laid larger eggs than did females of *C. septempunctata*. In 2005, egg volume did not differ between females that were provided prey consistently in the same amount (low or high food level) throughout the experimental period. Previous studies of predatory coccinellids also indicate that egg size does not vary with prey availability when individual females consume a consistent amount of food over time (Dixon and Guo 1993).

In general, females are predicted to invest more energy per offspring and lay larger eggs at low prey density (Clutton-Brock 1991; Roff 2002). However, larger egg volume was produced in response to greater prey availability in our experiment in 2006. In our experiment, food availability was increased over time, and such increasing prey availability may directly lead to increased investment in individual eggs along with an increase in general in egg production.

Differences in egg volume affected the survivorship of hatchlings. Hatchlings from the larger eggs of *C. transversoguttata* survived longer in the absence of prey than did hatchlings from the smaller eggs of *C. septempunctata*. Hatchling survival from eggs of both species, as laid by females provided with prey in excess, high, low, and intermittent daily amounts, was related to relative size of eggs. Thus, hatchlings from

eggs laid in the excess prey treatment survived longer on average in the absence of prey, than did hatchlings from the smaller eggs laid in the treatments of high, low and intermittent prey availabilities. A similar relationship between egg volume and hatchling survivorship (i.e., hatchlings from larger eggs survive longer than those from smaller eggs) was reported by Gilbert (1990) and Solbreck et al. (1989). Perhaps females were unable to compensate fully to low prey availability in producing eggs with sufficient yolk. Thus, within a species, larger eggs may promote greater hatchling survival than do smaller eggs laid under stressful food conditions.

A previous study of aphidophagous lady beetles did not find a relationship between the average size (weight) of eggs laid at various prey densities and the survivorship of hatchlings (Dixon and Guo 1993). Eggs were selected for the hatchling survivorship experiment in the days immediately following the switch from supplying aphids in excess to reduced amounts each day. In contrast, Dixon and Guo (1993) selected eggs from females that were each maintained throughout the experiment at a constant, designated level of prey availability. Differences in the outcomes of the two studies may be the result of these differences in experimental design.

Interesting differences in levels of egg viability also occurred among treatments in the experiments presented. In particular, eggs were less viable when laid by females provided with low versus high numbers of aphids each day. Perry and Roitberg (2005) also reported high numbers of infertile eggs associated with low prey availability in another predatory lady beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). Such results suggest the possibility that females control the level of egg fertility (and thereby viability) in response to prey density (e.g., Osawa 1992; Perry and Roitberg 2005).

In general, a large proportion of lady beetle eggs are apparently infertile; i.e., they do not develop into an embryo, and instead are consumed by larvae that hatch from the same egg batch (Hodek and Honek 1996; Dixon 2000). These eggs have been hypothesized to be trophic eggs, reflecting a maternal strategy to provide food for newly hatched offspring (Osawa 1992; Perry and Roitberg 2005). Trophic eggs usually differ from viable eggs, in that they lack micropyles as described by Chapman (1998). Polis (1981) found widespread evidence of oophagy in many arthropods, including mostly predatory species such as spiders, social Hymenoptera (ants, wasps, and bees), coccinellid beetles and lacewings. He reported that 5–97% of a clutch may be trophic eggs, i.e., unfertilized, non-viable eggs that are used as a food supply by hatchlings. Although infertile eggs in the present study were not examined for absence of micropyles, it is possible that these eggs were produced as trophic eggs by females of *C. septempunctata* and *C. transversoguttata* in response to low prey availability. Alternatively, production of infertile eggs at low prey availability may reflect direct effects of food stress in reducing the ability of females to produce fertile eggs.

Prey or host populations in nature are usually spatially and temporally heterogeneous; therefore it is important to examine how predators such as lady beetles adjust constantly to such a dynamic environment to ensure their survival and the successful development of their progeny. The results of the experiments presented here demonstrate plasticity in responses of females of both *C. septempunctata* and *C. transversoguttata* to varying prey availability, as measured by wet weight, egg production, and egg size and viability. Although females of the two species responded similarly overall to varying prey availability in their rates of egg production, females of

C. septempunctata appeared especially capable of maintaining body weight while doing so.

Perhaps related to different abilities of the two lady beetle species to maintain body weight when prey are limited, another clear difference was apparent in both years in that field-collected *C. septempunctata* females produced more eggs when provided with prey in the laboratory than *C. transversoguttata* females did. This occurred throughout the entire experimental period in 2005, and during the initial two weeks (when females were provided with excess aphids) in 2006. Similarly higher rates of egg laying were also found for *C. septempunctata* females in a field study (Chapter 2). These results suggest that overwintered *C. septempunctata* and *C. transversoguttata* females differ in physiological condition in alfalfa fields in early spring when aphid numbers are low. This may reflect differences in foraging success not only during the spring in the alfalfa fields from which females were collected, but also during the previous summer. Thus, overwintering lady beetles may use remaining fat reserves from the previous year to enhance reproduction upon spring emergence (see Dixon 2000, p. 29). Because *C. transversoguttata* has stronger tendency than *C. septempunctata* to produce more than a single generation per year (Obrycki and Tauber 1981; Chapter 2), females of *C. transversoguttata* that will overwinter may have less time over the summer and autumn to forage, and hence they may enter the winter with fewer fat reserves than females of *C. septempunctata*. Consequently, they may emerge in poorer physiological condition the following spring, and may remain so when foraging in alfalfa fields with low aphid numbers.

In summary, the introduced lady beetle *C. septempunctata* reproduces with

greater success in spring alfalfa fields of northern Utah than does the native species *C. transversoguttata* that it is displacing (Chapter 2). The results of the experiments presented here do not support the hypothesis that this greater success arises because the introduced species, in comparison to the native species, converts relatively more aphid biomass into eggs when females of these two species consume pea aphids at low rates. However, the results do support the hypothesis that females of the invasive species have ability to produce, more smaller eggs by trade-offs between egg number and size, and have greater ability to maintain their body mass at low aphid density, such as frequently occurs in the spring in alfalfa fields of northern Utah. Such ability may enhance the reproductive output of females as aphids become more numerous (e.g., Evans and Gunther 2005). Further studies are needed to determine the degree to which differing physiological condition of overwintered, introduced and native females results from differences in fat reserves upon spring emergence and/or from foraging success thereafter in spring alfalfa. Such studies may provide insight ultimately as to why the introduced *C. septempunctata* so rapidly has become the dominant aphidophagous lady beetle in this habitat.

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Table 4-1 Results of two-way ANOVA of wet weight and egg production of *Coccinella septempunctata* and *C. transversoguttata* in each experimental period of the second experiment, for four levels of food provided (excess, high, low, and intermittent).

	<i>Wet weight</i>			<i>Egg production</i>		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>1st period</i>						
Species	1, 76	6.64	0.0119	1, 76	3.21	0.077
Treatments	3, 76	5.19	0.0026	3, 76	69.26	<0.0001
Species x Treatment	3, 76	0.81	0.4949	3, 76	0.81	0.4919
<i>2nd period</i>						
Species	1, 67	3.19	0.0786	1, 67	0.32	0.5724
Treatments	3, 67	3.45	0.0214	3, 67	65.52	<0.0001
Species x Treatment	3, 67	1.30	0.2821	3, 67	1.44	0.2403
<i>3rd period</i>						
Species	1, 63	8.95	0.0040	1, 63	2.06	0.1559
Treatments	3, 63	2.69	0.0536	3, 63	31.39	<0.0001
Species x Treatment	3, 63	2.76	0.0492	3, 63	2.07	0.1132

Table 4-2 Results of two-way ANOVA of egg volume, total egg volume and egg viability of *Coccinella septempunctata* and *C. transversoguttata* in each experimental period of the second experiment, for four levels of food provided (excess, high, low, and intermittent).

	Egg volume			Total egg volume			Egg viability		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>1st period</i>									
Species	1, 54	46.17	<0.0001	1, 56	4.04	0.0492	1, 59	11.81	0.0011
Treatments	3, 54	13.64	<0.0001	3, 56	46.18	<0.0001	3, 59	5.61	0.0019
Species*Treatment	3, 54	0.18	0.9077	3, 56	1.21	0.3144	3, 59	0.83	0.4825
<i>2nd period</i>									
Species	1, 57	19.08	<0.0001	1, 61	0.73	0.3963	1, 58	10.11	0.0024
Treatments	3, 57	10.06	<0.0001	3, 61	36.35	<0.0001	3, 58	2.90	0.0427
Species*Treatment	3, 57	0.82	0.4870	3, 61	1.05	0.376	3, 58	1.76	0.1647
<i>3rd period</i>									
Species	1, 53	7.72	0.0075	1, 62	0.18	0.6742	1, 53	10.19	0.0024
Treatments	3, 53	2.20	0.0993	3, 62	30.61	<0.0001	3, 53	2.15	0.1052
Species*Treatment	3, 53	1.24	0.3059	3, 62	1.09	0.3598	3, 53	0.25	0.8606

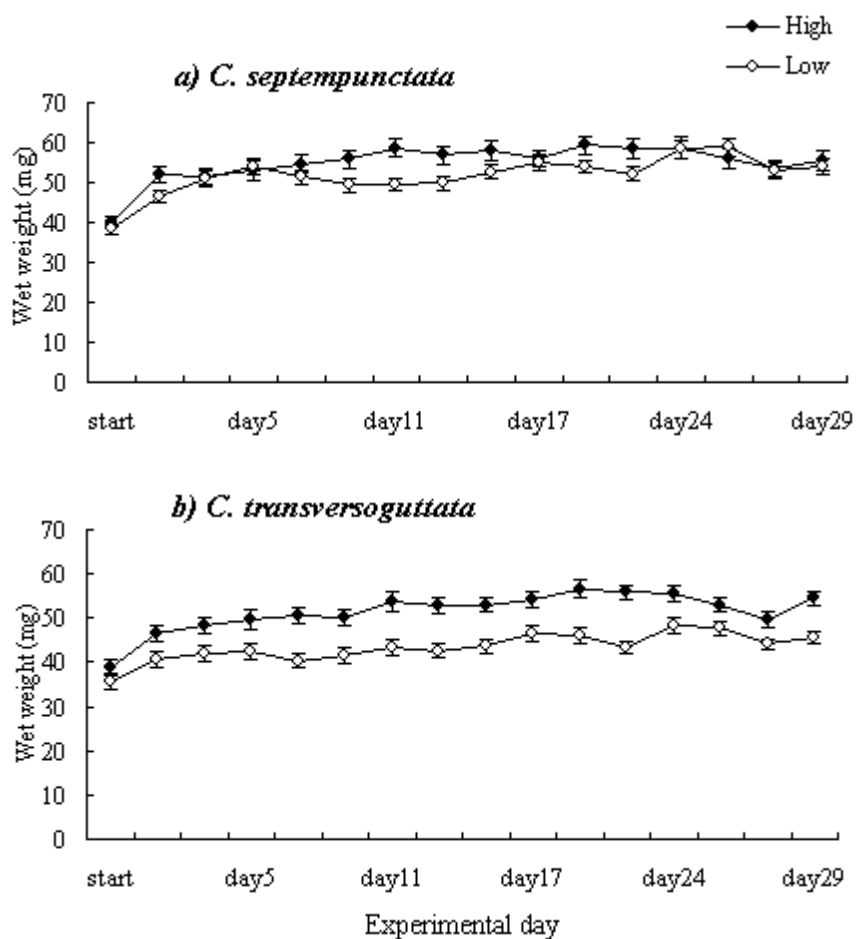


Fig. 4-1 Mean wet weight (\pm SE) of females of (a) *Coccinella septempunctata* and (b) *C. transversoguttata* provided with high and low numbers of aphids for 30 days (first experiment).

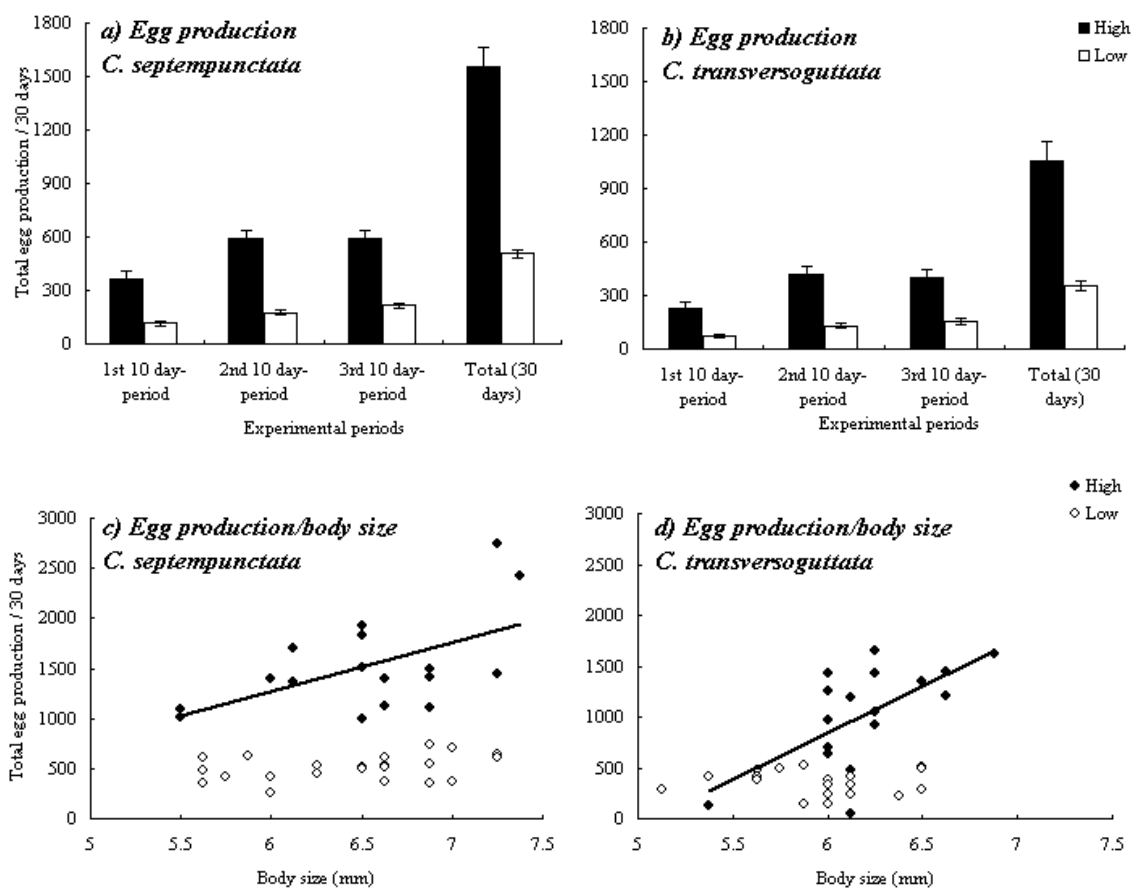


Fig. 4-2 Mean total number of eggs (\pm SE) produced by females of (a) *Coccinella septempunctata* and (b) *C. transversoguttata* provided with high and low numbers of aphids, for each 10-day period and for the full 30 days (first experiment). Regression of egg production for the full 30 days against female body size (elytron length in mm) for (c) *C. septempunctata* and (d) *C. transversoguttata* provided with low numbers ($P > 0.1$, for both species) or high numbers of prey (*C. septempunctata*: $Y = 488X - 1659.9$, $adjR^2 = 0.26$, $P = 0.02$; *C. transversoguttata*: $Y = 922X - 4690.2$, $adjR^2 = 0.36$, $P = 0.006$).

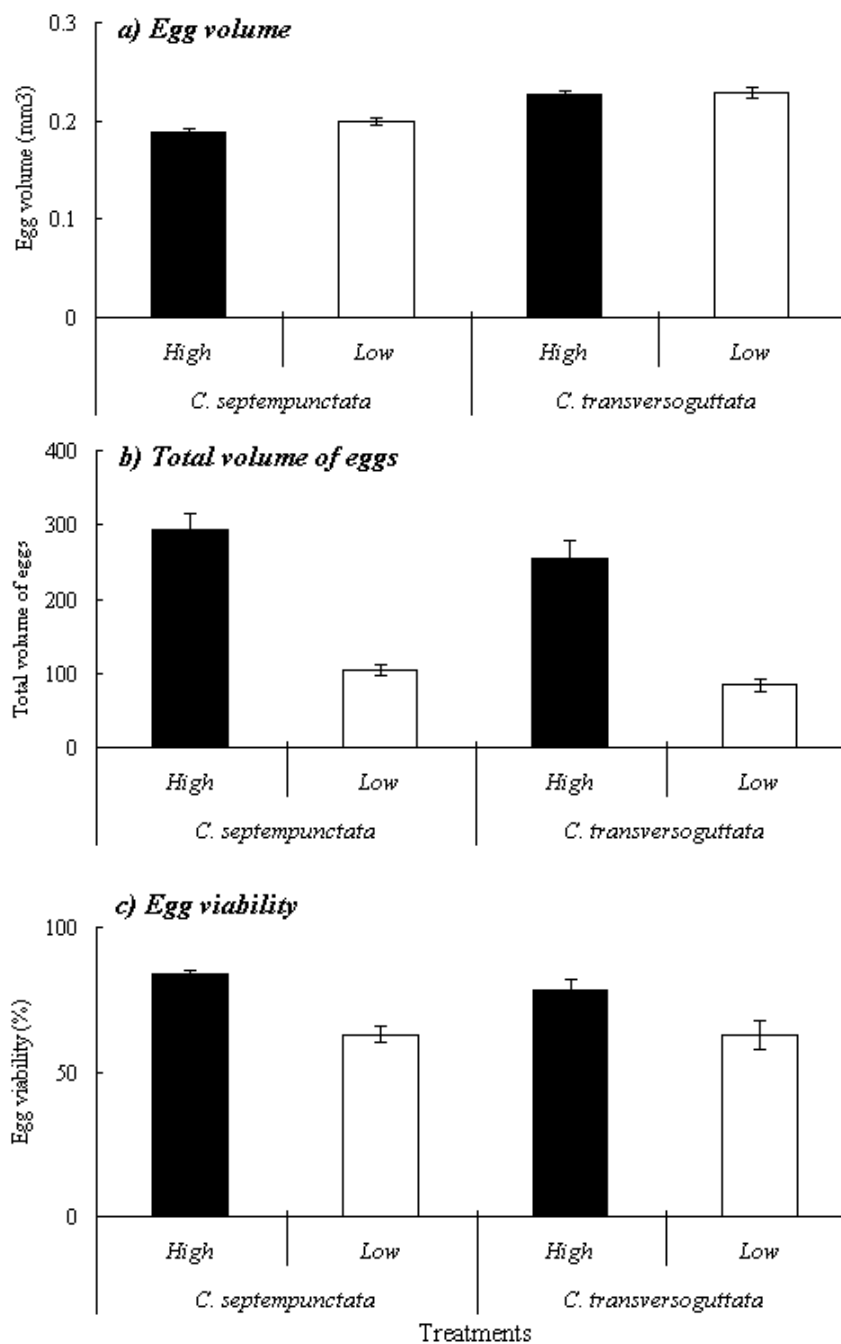


Fig. 4-3 (a) Mean egg volume (mm³) (\pm SE), (b) mean total volume of eggs (\pm SE), and (c) mean percent egg viability (\pm SE), for females of *Coccinella septempunctata* and *C. transversoguttata* when provided with high or low numbers of aphids for 30 days.

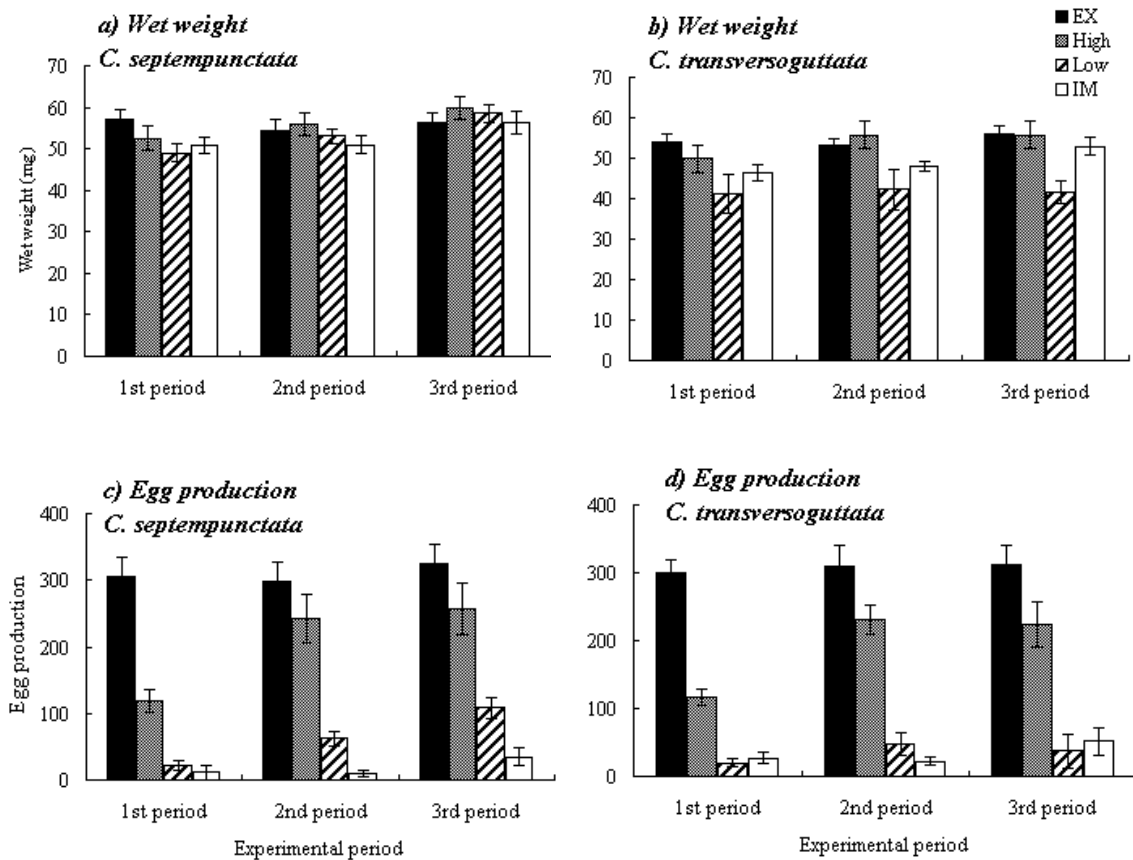


Fig. 4-4 Mean wet weight (\pm SE) of females of (a) *Coccinella septempunctata*, and (b) *C. transversoguttata*, and mean total number of eggs (\pm SE) produced by females of (c) *C. septempunctata*, and (d) *C. transversoguttata*, when provided with aphids in excess (EX), or in high, low, or intermittent (IM) numbers, during each experimental period of the second experiment.

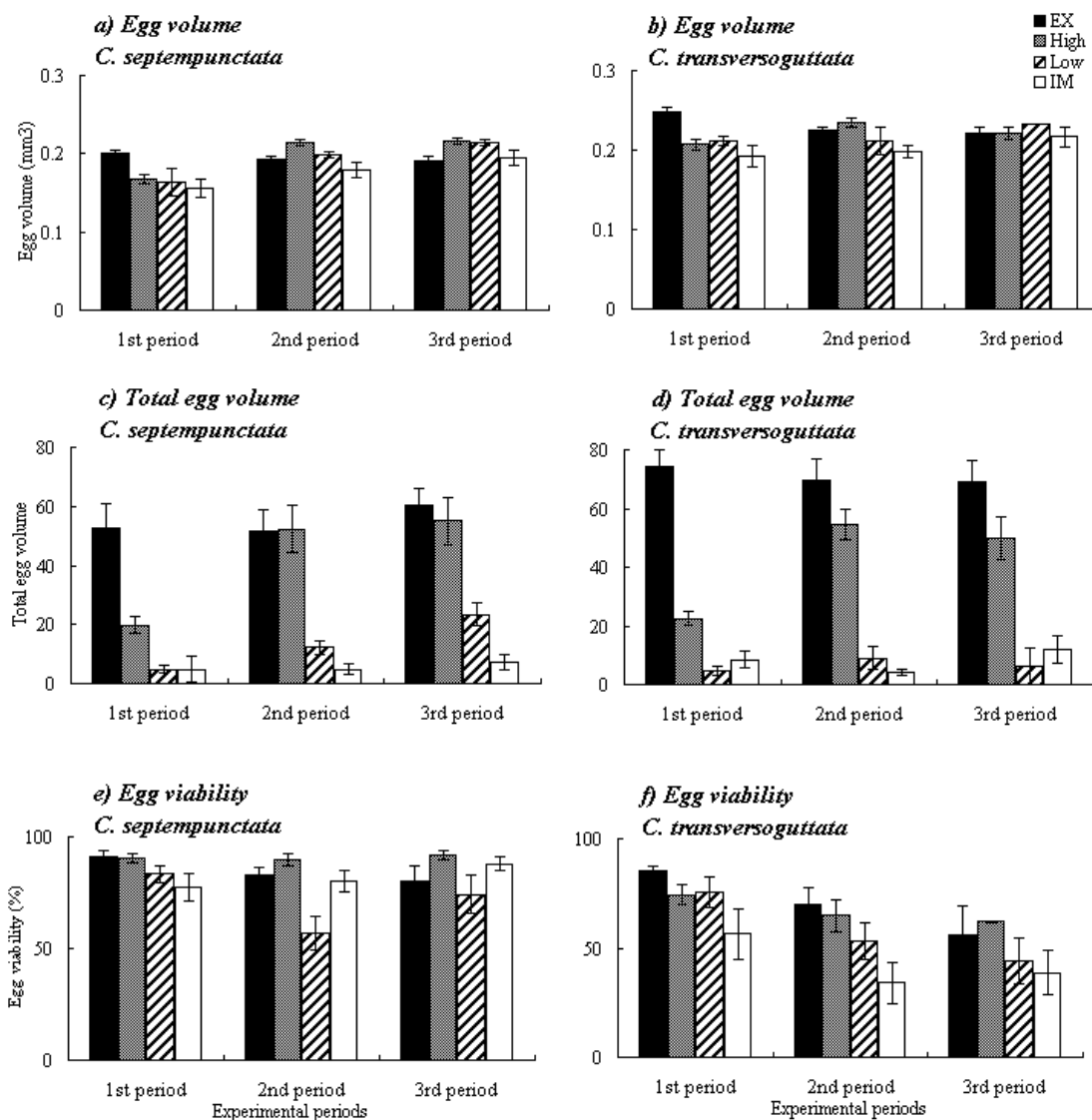


Fig. 4-5 Mean egg volume (mm^3) (\pm SE) produced by females of (a) *Coccinella septempunctata*, and (b) *C. transversoguttata*, mean total volume of eggs (\pm SE) for (c) *C. septempunctata* and (d) *C. transversoguttata*, and mean percent egg viability (\pm SE) for (e) *C. septempunctata* and (f) *C. transversoguttata*, when provided with aphids in excess (EX), or in high, low, or intermittent (IM) numbers, during each experimental period of the second experiment.

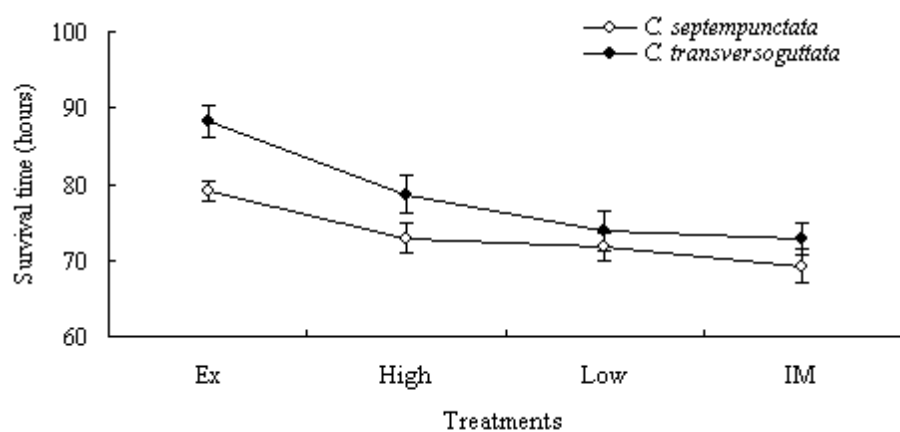


Fig. 4-6 Survivorship of hatchlings from eggs laid by females of *Coccinella septempunctata* and *C. transversoguttata* that were provided with aphids in excess (EX), or in high, low, or intermittent (IM) numbers in the second experiment.

CHAPTER 5

OVARIAN DEVELOPMENT AND OBSERVATION OF OOSORPTION IN TWO
PREDATORY LADY BEETLES (COLEOPTERA: COCCINELLIDAE)

Abstract Ovarian and egg-laying dynamics of two species of lady beetle, *Coccinella septempunctata* and *C. transversoguttata*, were examined here in two laboratory experiments in which aphid prey first were provided in excess for an extended period and then were removed. The results of the experiments presented here show that females of *C. septempunctata* respond more quickly by reducing their reproductive effort than females of *C. transversoguttata* to removal of prey in an experimental setting. Data from dissection showed the potential occurrence of oosorption in both species; however, no clear differences between the two species were observed. In *C. septempunctata*, about 5 potential eggs (i.e., mature oocytes) disappeared in studies performed in 2006 and 2007, and in *C. transversoguttata*, an average of about 4 and 1.8 potential eggs disappeared in 2006 and 2007 studies, respectively, 2 to 3 days after prey removal. In one study, aphids were provided again to experimental females after 6 days during which the prey were withheld. Females of both species started to reproduce 2 to 3 days after they again were provided with aphids in excess. Females of *C. septempunctata* laid almost the same number of eggs as they had before they were starved; however, previously starved *C. transversoguttata* females produced fewer eggs than they had before. Such immediate adjustment of reproductive effort when prey were removed and high recovery of reproductive rate when prey again became available, as observed in *C. septempunctata*, may contribute to the relative success of *C. septempunctata* in reproducing in alfalfa fields.

Introduction

The potential for trade-offs between current and future reproduction is an intriguing issue in theories of life-history evolution because limited resources must be allocated among growth, maintenance and reproduction (e.g., Pianka 1981). For insect predators such as lady beetles, prey populations are ephemeral and vary greatly in size over space and time. Therefore, females need to forage widely and adjust constantly to such dynamic environments to ensure their survival, reproduction and success of progeny. They may do so by adjusting their current reproductive effort. For example, females may reduce rapidly the number and size of eggs laid when prey become unavailable. Also, oosorption is well known as an “egg recycling strategy” for ensuring future reproductive success, in which the nutrients in maturing oocytes within a female’s reproductive tract are withdrawn and reallocated to somatic tissues as gravid females choose not to lay eggs in response to internal and/or external (environmental) factors (e.g., in response to nutritional deficiency, lack of ovipositional sites, and so forth; Bell and Bohm 1975; Papaj 2000). Oosorption occurs widely among many orders of Insecta (e.g., Bell and Bohn 1975). Oosorption can be a key element in the trade-off between current reproduction and survival (e.g., Bell and Bohm 1975; Ohgushi and Sawada 1985; Ohgushi 1996).

Aphidophagous lady beetle adults require aphids as food to maintain themselves and to stimulate reproductive activity (e.g., Evans and Dixon 1986; Richards and Evans 1998). However, it is not well understood whether lady beetles engage in oosorption of mature oocytes. Recently, Osawa (2005) reported the occurrence of oosorption in *Harmonia axyridis* (Coleoptera, Coccinellidae). To understand the ovarian dynamics and

reproduction of two species of lady beetles, *Coccinella septempunctata* and *C. transversoguttata*, and to determine whether oosorption occurs in either or both, laboratory experiments were conducted in which females were provided prey in excess initially and then the prey (aphids) were removed. These two species are interesting to compare because the introduced species *C. septempunctata* has become the dominant lady beetle species in alfalfa fields in western North America and has displaced the native species *C. transversoguttata*. Aphid numbers can vary from very high to very low in these alfalfa fields, often over short spans of time. Therefore, the general hypothesis is tested here that females of *C. septempunctata* may respond more quickly in modifying their reproductive effort than females of *C. transversoguttata*, in response to changes in prey conditions in an experimental setting. Such a difference between species could be related, furthermore, to the tendency of well-fed *C. septempunctata* females to lay more, individually smaller eggs than females of *C. transversoguttata* (see Chapters 2 and 4).

Materials and Methods

Two experiments (one each in 2006, and in 2007) were conducted with adults of *Coccinella septempunctata* and *C. transversoguttata* that were collected from alfalfa fields near Logan, Utah, in early spring (late April to early May). These adults had recently arrived in the alfalfa fields from overwintering sites and were not yet fully active reproductively (see Chapter 3).

In 2006, the adults were placed in an incubator at 18°C, 16L:8D, with a tube of sugar water, and held for 2 to 3 weeks before being used for the experiment at first, and then transferred for the experiment to the 22°C incubator. In this period prior to the

experiment, adults were held as groups of females or males. 60 and 65 mating pairs of *C. septempunctata* and *C. transversoguttata*, respectively, were created from this group of adults, and were provided with pea aphids in excess daily for 15 days (i.e., until most females had become fully active reproductively, and were laying eggs at or near maximum rates). The aphids were removed at the start of the sixteenth day, and the pairs of lady beetles were held without prey for the next 6 days (they were provided only with sugar water). Females from ten pairs of each species were sacrificed at the end of the first day without prey (Day 16) and dissected to assess reproductive condition. Similarly, as many as 10 females of each species were sacrificed and dissected at the end of each subsequent day that they were held without prey (i.e., on Days 17–21; fewer than 10 pairs were dissected beginning on Day 18, as dictated by mortality in the absence of prey, with a minimum of 3 females dissected for *C. transversoguttata* at the end of Day 21). As explained in more detail below, dissection methods and evaluation of reproductive (ovarian) condition followed Kurihara (1975) and Phoofolo et al. (1995). Throughout the entire experiment (i.e., both with prey present in excess for the first 15 days, and with prey absent for 6 days thereafter), the number of eggs laid by each female on each day was recorded. Data from females that died during the experiment (other than those sacrificed), or that laid few or no eggs when provided with excess prey, were excluded. Final sample sizes were 58 pairs for *C. septempunctata*, and 38 pairs for *C. transversoguttata*.

In 2007, adults collected from alfalfa fields were used for the experiment immediately upon collection. In total, 114 and 120 mating pairs of *C. septempunctata* and *C. transversoguttata*, respectively, were created for the experiment. As in 2006, pairs

were offered pea aphids in excess daily for 15 days. Beginning on the sixteenth day, aphids were removed from 104 pairs of *C. septempunctata*, and 108 pairs of *C. transversoguttata* (the pairs were provided with only sugar water). The remaining 10 pairs of *C. septempunctata*, and 12 pairs of *C. transversoguttata*, were provided with excess pea aphids continuously throughout the entire experimental period. Reproduction was compared between pairs with and without prey. Females from 12 pairs of each species were sacrificed at the end of the first day without aphids present (Day 16), and dissected to assess reproductive condition. Similarly, as many as 12 females of each species were sacrificed and dissected at the end of each subsequent day that they were held without prey (e.g., on Day 19, 11 females were dissected as determined by mortality in the absence of prey). The methods of dissection and evaluation of reproductive condition were the same as those used in 2006. Pairs without prey for 6 days that were not sacrificed (i.e., 26 pairs of *C. septempunctata* and *C. transversoguttata* in which the female survived the 6-day period) were provided pea aphids again in excess daily, beginning on the twenty-second day.

For both experiments, pairs were held individually in Petri dishes (5.7cm in diameter and 1.5cm in height) in an incubator at 22°C, 16L:8D, for the experimental period. On each day of the experiment, each pair of lady beetles was transferred to a new Petri dish provisioned with an excess amount of pea aphids [reared on fava bean, *Vicia faba* L. (Fabaceae), in the greenhouse]. One drop of water was also added on the lid to keep the humidity inside of the Petri dish. A strip of folded filter paper was put in each Petri dish, both to create more oviposition sites for a female and to minimize egg cannibalism by males and their disturbance of ovipositing females. Females that died

during experiments were not replaced, and as noted above, data for these females were removed before analyses. In both years, however, males that died during the experiment were replaced, such that females remained paired with a male throughout the experiment.

Wet weights of females were measured every fifth day during days when adults were provided pea aphids in excess, and every day during the period without aphids. The number of eggs produced by each female was recorded at the end of each 24 h period (i.e., at the time that pairs were transferred to new Petri dishes each morning). Egg cannibalism occurred only rarely. When egg cannibalism was observed, the residues of eggs could be identified, and cannibalized eggs were added into counts of daily egg production. Especially during the period without aphids, egg production was checked and eggs were removed from the Petri dish every 4 h to prevent the occurrence of egg cannibalism.

On days when lady beetles were not fed, as well as on the day before prey were removed (i.e., Days 15 to 21 during both experiments), the length and width of five randomly selected eggs per female were measured using a SZX Olympus stereomicroscope at 30 x magnification, for those females that laid eggs. Egg volume (mm^3) was calculated from these measurements, following Phoofolo et al. (1995) and Richards and Evans (1998), by using the formula for a prolate spheroid, $(4/3)\pi ab^2$, where a is equal to half the egg length and b is equal to half the egg width. A mean egg volume was then calculated for each female.

Egg viability was measured on Days 15–21. One egg batch was taken from each female that produced eggs, and put in an incubator for several days. The emergence of hatchlings was checked every 4 to 6 h (to prevent sibling egg cannibalism, hatchlings

were removed immediately). From these observations, the overall percentage of eggs laid by each female that were viable was calculated.

Females sacrificed for dissection were frozen each morning after egg production and wet weight had been measured. The three most developed egg follicles in ovarioles from each ovary were measured for length and width (for a total of 6 follicles per females). The number of mature oocytes, recognized by their fully developed shape, that were found in lateral oviducts (associated with right and left ovaries) was also counted.

Data analyses

The wet weights of *C. septempunctata* and *C. transversoguttata* females at the beginning of the experiment, and the averages of weights on Days 10 and 15 (during the 15-day period in which females were provided with aphids in excess), were analyzed by paired t tests for each species. The wet weights of females thereafter, on Days 15 to 21 (i.e., including the period when aphids were not provided from Days 16–21), were analyzed by repeated measures of analysis of variance (ANOVA), with square root data transformation (Day 15 was included for pre- and post-aphid removal comparison). For the experiment in 2007, an additional repeated measures of ANOVA with square root data transformation was conducted for wet weights on Days 22–31, to examine responses of females when aphids again were provided in excess.

As a measure of maximal reproductive rate when excess prey was available, the mean number of eggs per day produced by females of *C. septempunctata* and *C. transversoguttata* on Days 13–15 was calculated. Following transformation by square root, means were compared between species by one-way ANOVA. Egg production on

Day 15 (with aphids provided in excess), and during the period in which aphids were removed (Days 16, 17, and 18), was analyzed by repeated measures of ANOVA with square root transformation (Day 15 was included for comparison of pre- and post-aphid removal).

For the 2007 experiment, daily egg production during Days 24 to 31 was analyzed by repeated measures of ANOVA with square root data transformation. Two-way ANOVA was also used to compare egg production during Days 13–15 and Days 29–31 by females of the two species with or without the prior experience of having aphids removed for 6 days. Further two-way ANOVA was conducted to compare the rate of egg production recovery during Days 29–31 compared with egg production during Days 13–15 (egg production during Days 29–31 / egg production during Days 13–15) for females of the two species, with or without the prior experience of having aphids removed for 6 days. Data were transformed by square root.

Egg volume and egg viability, for eggs produced on Days 15–18, were analyzed by two-way repeated measures of ANOVA (year x species, with days as repeated measures). The number of mature oocytes found in lateral oviducts (i.e., the total number from left and right lateral oviducts combined), and the mean size of the three most developed follicles in the ovarioles of left and right ovaries were analyzed by three-way ANOVA (year x species x day).

Results from ovarian dissections were combined with results for numbers of eggs laid daily to examine the dynamics of egg production over the next 6 days when food was withdrawn on Day 16. Mature oocytes found in the oviducts by dissection at the end of

the first 24 h without aphids (i.e., at the end of Day 16) were considered as potential eggs, which could be laid by females during the next 24 h (i.e., on Day 17).

To explore whether females continued to produce additional mature oocytes, or resorbed previously produced mature oocytes, following the removal of prey, net egg production (i.e., net production of mature oocytes, including those laid as eggs) was examined and compared between the two species. For these comparisons, the following three means for individual females were considered: the number of mature oocytes found by dissection in a female's oviduct (1) at the end of a given day (e.g., Day 16), and (2) at the end of the next day (e.g., Day 17), and (3) the number of eggs laid during the next day (e.g., Day 17). Positive net egg production occurred when the sum of (2) + (3) was greater than (1), and negative net egg production occurred when this sum of (2) + (3) was less than (1). Three-way ANOVA (species \times year \times day [Day 16 vs 17]) was used for this analysis.

Similarly, the number of potential eggs found at dissection on Day 17 (i.e., 48 hours after the removal of prey) was compared with the sum of (a) the number of potential eggs 24 h later plus (b) the number of eggs laid between these two times (i.e., during Day 18), again by three-way ANOVA. Data were log transformed. Further one-way ANOVA was conducted for the number of eggs laid during Day 17 versus the number of mature oocytes in the oviduct at the end of Day 16 for *C. septempunctata* in 2007, and for *C. transversoguttata* in both years, to examine whether all of the mature oocytes present in the oviduct at the end of Day 16 were laid over the next 24 h (Day 17). The number of mature oocytes between Day 18 and 19 for *C. septempunctata* in 2006 was also compared by one-way ANOVA.

To assess further the overall tendency of *C. septempunctata* and *C. transversoguttata* females to resorb rather than lay mature eggs following removal from prey, the following index was calculated for each of the females sacrificed on Day 21 (i.e., 6 days after prey removal). The total numbers of eggs laid by these females during the final 5 days without food (Days 17 to 21) was divided by the sum total of counts of mature eggs in the oviduct of a female of that species on each of the 6 days without food (i.e., sum of the mean number of mature oocytes for each day during Days 16 to 21). The data were analyzed both by combining results from the two years for each species (one-way ANOVA), and by distinguishing between results from the two years (two-way ANOVA with year x species).

All of the analyses were performed by using SAS 9.1 (SAS Institute 2002–2003). Repeated measures analyses in Proc Mixed with un-structure model (including determination of degrees of freedom as calculated in SAS by the Kenward-Roger method) were used for these analyses.

Results

In both experiments, females of both species gained weight during the initial 15 days, when they were provided with pea aphids in excess (Fig. 5-1a,b; paired t test for weight on Day 1 versus the average of weights on Days 10 and 15 for *Coccinella septempunctata* in 2006: $t_{57}=-19.63$, $P<0.0001$, and in 2007: $t_{112}=-18.74$, $P<0.0001$; and for *C. transversoguttata* in 2006: $t_{62}=-7.81$, $P<0.0001$, and in 2007: $t_{118}=-23.54$, $P<0.0001$).

Thereafter, when aphids were removed, females initially lost weight and then stabilized at lower weights during the period without prey (Fig. 5-1a,b). For both experiments (2006 and 2007), repeated measures of ANOVA of female weight on Days 15–21 (with wet weight on Day 15 included for comparison of pre- and post-aphid removal) yielded a significant effect of time for both *C. septempunctata* and *C. transversoguttata* (2006: $F_{6,2.77}=9.29$, $P=0.05$; 2007: $F_{6,104}=131.66$, $P<0.0001$), as well as for species (2006 $F_{1,84.3}=4.8$, $P=0.03$; 2007: $F_{1,214}=19.46$, $P<0.0001$) with *C. septempunctata* females weighing more on average than females of *C. transversoguttata*. The interaction of time with species was not significant in either year (2006: $F_{6,2.77}=2.34$, $P=0.27$; 2007: $F_{6,104}=1.87$, $P=0.09$).

In 2007, females of *C. septempunctata* and *C. transversoguttata* were provided pea aphids again beginning on Day 22, and their body weights increased (Fig. 5-1b). Repeated measures of ANOVA for weights on days 22–31 revealed significant effects of species ($F_{1,91.7}=11.69$, $P=0.0009$) and time ($F_{6,67.4}=43.47$, $P<0.0001$) on weight; however, there was not a significant interaction of species with time ($F_{6,67.4}=0.84$, $P=0.54$). In general, the responses of females of *C. septempunctata* and *C. transversoguttata* were similar in regard to their changes in weight, both when aphids were removed and when aphids were subsequently made available again to the lady beetles.

After sustained feeding on excess prey, females of *C. septempunctata* produced more eggs than did females of *C. transversoguttata* (Fig. 5-1c,d; one-way ANOVA of average daily egg production on Days 13–15 in 2006: $F_{1,93}=67.37$, $P<0.0001$; and in 2007: $F_{1,215}=49.19$, $P<0.0001$). Egg production decreased for both species when aphids were removed (repeated measures of ANOVA of daily egg production on Days 15–18,

effect of time: 2006 $F_{3,93}=83.14$, $P<0.0001$; 2007 $F_{3,196}=197.21$, $P<0.0001$), although the pattern of decrease with time differed between the two species (interaction of species with time, 2006: $F_{3,93}=7.96$, $P<0.0001$; 2007: $F_{3,196}=10.22$, $P<0.0001$). In both years, females of *C. septempunctata* reduced their rate of egg laying immediately in response to the removal of prey (i.e., on Day 16), but females of *C. transversoguttata* did not do so until one day later (i.e., on Day 17) (Fig. 5-1c,d). Neither species laid eggs on Days 20 and 21 (the fifth and sixth days without prey).

When pairs were provided pea aphids again in 2007 beginning on Day 22, females of both species began producing eggs again soon thereafter, with the first eggs laid by most females on Day 25 (Fig. 5-1d). Females of *C. septempunctata*, however, produced more eggs than *C. transversoguttata* (repeated measures of ANOVA for daily egg production on Days 24–31: effect of species $F_{1,66.7}=20.20$, $P<0.0001$; effect of time $F_{7,60.5}=13.08$, $P<0.0001$, Fig. 5-1d). There was not a significant interaction effect of species with time ($F_{7,60.5}=1.19$, $P=0.32$).

Mean egg production during Days 13–15 versus Days 29–31 was compared for females of both species with the experience of 6 days (Days 16–21) without aphids (Fig. 5-2). Females of *C. septempunctata* produced more evenly matched numbers of eggs as they had before aphids were removed (i.e., on Days 13–15), than did females of *C. transversoguttata* (Fig. 5-2a, two-way ANOVA for mean daily egg production between Days 13–15 and Days 29–31 (time effect) for females that experienced 6 days without aphids: effect of species $F_{1,89}=42.48$, $P<0.0001$; effect of time $F_{1,89}=1.02$, $P=0.32$; interaction of species x time $F_{1,89}=4.18$, $P=0.04$).

Mean egg production during Days 13–15 and Days 29–31 by females of both species without the experience of 6 days without aphids (i.e., with continuous feeding and reproduction throughout the entire experimental period) found that *C. septempunctata* produced more eggs than *C. transversoguttata* (Fig. 5-2b, two-way ANOVA: effect of species $F_{1,28}=21.84$, $P<0.0001$), and there was a significant effect of time ($F_{1,28}=8.08$, $P=0.008$). There was not a significant interaction of species with time ($F_{1,28}=0.08$, $P=0.78$).

The rate of egg production during Days 29–31 compared with the egg production during Days 13–15 were analyzed for females of the two species, with or without the prior experience of having aphids removed for 6 days (Fig. 5-2c). Females of *C. septempunctata* had significantly higher reproductive rate during Days 29–31 than *C. transversoguttata* (two-way ANOVA: effect of species $F_{1,58}=8.19$, $P=0.006$). Interestingly, females of both species with prior starvation had significantly higher reproductive rate than females without starvation for 6 days ($F_{1,58}=5.08$, $P=0.028$). No significant interaction effects of species with females with or without the prior experience of prey removal for 6 days was observed ($F_{1,58}=0.78$, $P=0.38$).

Females of *C. transversoguttata* laid larger eggs than did females of *C. septempunctata* throughout, including on the day before prey removal (i.e., Day 15) and on subsequent days (Days 16–18) when aphids were withheld (Fig. 5-3a,b and Table 5-1). Both species produced smaller and smaller eggs on successive days. The three-way interaction of year x species x day was not significant; however, there were significant interactions of species effect with day, and year with day, reflecting the relatively large eggs that were produced on Day 17 in 2006 by females of *C. transversoguttata*.

The percentages of eggs viability did not differ between the two species (Fig. 5-4 and Table 5-1). There were significant effects of year and day (i.e., day of experiment) in response to withholding of aphids: fertility was higher on average in 2007 than in 2006, but in both years, viability declined from Day 15 to Day 18 (Fig. 5-4). The interaction effect (year x species x day) was not significant; however, the interaction of species effect with day was significant, reflecting that differences in viability between the two species were inconsistent among days (Table 5-1).

The ovaries of females that were sacrificed at the end of each day on which aphids were not provided (Days 16–21) contained oocytes that differed widely in stage of development. Fewer mature oocytes were found in the lateral oviducts over time for both species (Fig. 5-5a,b, and Table 5-2). There was marginal effect of species (Table 5-2). In 2006, *C. septempunctata* females had more mature oocytes in their oviducts 24 h after prey removal (i.e., at the end of Day 16) than did *C. transversoguttata* females; however, the interaction of year x species x day was not significant.

Smaller and smaller egg follicles (i.e., oocytes that had not yet passed into the oviduct) were found within ovarioles in both species after the removal of aphids (Fig. 5-5c,d and Table 5-2). The decrease in follicle size over time happened especially rapidly in 2007 following removal of aphids, leading to a significant interactions of year x day. In addition, there was a significant three-way interaction of year x species x day (Table 5-2). This interaction reflected that follicle size tended to decrease more rapidly for *C. septempunctata* than for *C. transversoguttata* in both years, but was expressed later and most clearly in 2006 (Day 19 versus Day 18) than in 2007 (Day 18 versus Day 17) (Fig.5-5).

Positive net egg production on Day 17 was apparent for *C. septempunctata* in 2007, and for *C. transversoguttata* in both years (Fig. 5-6 and Table 5-3; the marginally significant interaction of species x year x day reflects that such positive net egg production was not readily apparent for *C. septempunctata* in 2006). These results indicate that during the second 24 h after prey removal (i.e., during Day 17), females continued to mature additional eggs in their reproductive tracts. Furthermore, the number of eggs laid during Day 17 did not differ significantly from the number of mature oocytes in the oviduct at the end of Day 16 for *C. septempunctata* in 2007, and for *C. transversoguttata* in either year (one-way ANOVA: $P > 0.1$ in each case), suggesting that all of the mature oocytes present in the oviduct at the end of Day 16 were laid over the next 24 h.

A similar analysis for Day 18 reveals that for *C. septempunctata* in 2007 and *C. transversoguttata* in both years, the pattern switched to negative net egg production (Fig. 5-6 and Table 5-3). These results indicate that during the third 24 h after prey removal (i.e., during Day 18), some mature oocytes were reduced in size (or resorbed) rather than being laid (however, it is also possible that females also could have matured some more, although fewer, eggs at the same time). Approximately 4.8 mature oocytes in *C. septempunctata* in 2007, and 4.2 and 1.6 mature oocytes in *C. transversoguttata* in 2006 and 2007, respectively, disappeared during the next 24 h (i.e., they could not be accounted for by the number of eggs laid over the next 24 h + the number of mature oocytes present at the end of the day; Fig. 5-6a,b).

Females of *C. septempunctata* in 2006 deviated from this general pattern, in that their net egg production appeared to be positive rather than negative on Day 18 (as

reflected in a marginally significant interaction of species x year x days; Table 3). They switched to net negative egg production on the following day [Day 19], when they also ceased laying eggs (Fig. 5-6a). In the absence of egg-laying, the numbers of mature oocytes that were present in these females' oviducts decreased during Day 19 (fewer mature oocytes were present at the end of Day 19 than at the end of Day 18; one-way ANOVA: $F_{1,13}=12.16$, $P=0.004$). Approximately 5 potential eggs per female disappeared (or were resorbed) during Day 19.

Females of *C. septempunctata* in 2007, and females of *C. transversoguttata* in 2006 and 2007, laid very few eggs on or after the fourth day following prey removal (i.e., Days 19–21). The small numbers of mature oocytes which were present in the oviduct decreased over time and likely reflects resorption.

For both years combined, the mean value of the index (the total numbers of eggs laid during the final five days without food divided by the sum total of counts of mature eggs in the oviduct on each of the 6 days without food) for *C. septempunctata* and *C. transversoguttata* was 0.43 ± 0.09 , and 0.46 ± 0.09 , respectively (one-way ANOVA: $F_{1,34}=0.03$, $P=0.86$). For each year considered separately, the mean value of this index was 0.27 ± 0.11 and 0.05 ± 0.05 in 2006, and 0.53 ± 0.12 and 0.56 ± 0.09 in 2007, for *C. septempunctata* and *C. transversoguttata*, respectively (two-way ANOVA: effect of species, $F_{1,32}=0.43$, $P=0.51$; effect of year, $F_{1,32}=6.98$, $P=0.01$; interaction of species with year, $F_{1,32}=0.70$, $P=0.41$). Reflecting the better condition of females in general in 2007, when they were provided immediately with excess aphids upon collection, the index values for both species were significantly higher in 2007 than in 2006.

For both lady beetle species, empty ovarioles were observed on Days 19, 20, and 21 when aphids were removed (Fig. 5-7). Several follicles with degraded yolks were also observed in other ovarioles at the same time.

Discussion

Ovarian condition and reproductive responses of *Coccinella septempunctata* and *C. transversoguttata* were examined here when prey (aphids) initially were provided in excess for an extended period, and then were removed. The results indicate that *C. septempunctata* females respond more quickly to removal of prey, by laying fewer eggs than previously, than did females of *C. transversoguttata*. This occurred in both experiments in 2006 and 2007. In contrast, females of *C. transversoguttata* produced as many eggs on Day 16 as on Day 15 (when prey was still provided in excess) in both 2006 and 2007, and did not reduce their rates of egg production until Day 17 (i.e., until 24 hours after prey were removed).

Females of both species started to lay eggs again on Days 24 and 25 in 2007, 2 to 3 days after prey were again provided following a 6-day period with prey. Upon resumption of egg-laying, females of *C. septempunctata* laid almost the same number of eggs each day as they had before aphids were removed, but females of *C. transversoguttata* laid fewer eggs after versus before the period without prey. The mechanisms behind these differences between *C. septempunctata* and *C. transversoguttata* are unclear. However, it appears that *C. septempunctata* may have greater ability to adjust its reproductive output to fluctuation in prey availability.

In previous studies (Chapters 2 and 4), *C. septempunctata* females weighed more, and produced more and individually smaller eggs than did *C. transversoguttata* females. Results from the two experiments presented here are consistent overall with these previous results. Furthermore, in general, females of the two species responded similarly in their wet weight, egg volume, the number and sizes (volume) of mature oocytes, and egg viability, in response to the removal of prey (as noted in the Results section, there were a few exceptions to this generalization). Egg viability decreased during the period in which prey were withheld from females. However, even during Day 18 (and Day 19 in 2007 for *C. transversoguttata*), females laid viable eggs, although the number laid was small.

It appears that oosorption occurred in both species; however, overall, there were not clear indications from dissections that the two species differed in their patterns of oosorption. Females of *C. transversoguttata* in both years and of *C. septempunctata* in 2007 continued to produce mature oocytes on the second day (Day 17) without prey. In both years, the net egg production then became negative (i.e., counts of mature oocytes declined more than could be accounted for by egg-laying) on Days 18 and 19, as several potential eggs (mature oocytes) per female disappeared in both species. In *C. septempunctata*, about 5 potential eggs per female disappeared on Day 19 in 2006, and on Day 18 in 2007. In *C. transversoguttata*, about 4, and 1.8 potential eggs per female disappeared on Day 18 in 2006 and 2007, respectively. Because no egg-laying occurred on Days 20 and 21, it seems likely also that the mature oocytes present in the oviduct on those days were resorbed. An index (the ratio of the number of eggs laid to the sum of counts of mature oocytes) suggest that for the two years combined, as many as 54% to

57% of mature oocytes of both species were resorbed during the starvation period. Active oosorption following 2 to 3 days without aphids is suggested also by the empty ovarioles and follicles with degraded yolk that were observed upon dissection of sacrificed females of both species on Days 19, 20, and 21.

In parasitoid wasps, host deprivation and starvation cause immediate occurrence of oosorption (Rivero-Lynch and Godfray 1997; Heimpel and Rosenheim 1995; Heimpel et al. 1997; Jervis et al. 2000). Our data similarly indicate that oosorption begins soon (2 to 3 days) after the lady beetle females stop consuming prey. Another aphidophagous lady beetle, *Harmonia axyridis*, showed signs of oosorption even more rapidly (within 24 h of prey removal) (Osawa 2005). The comparison between the potential eggs and the net egg production allowed us to calculate how many mature oocytes “disappeared” rather than being laid. Females that were subjected to an extended period without prey subsequently laid more eggs than did females that continued to feed throughout the experiment on aphids provided in excess. This was true for both species, but especially true for *C. septempunctata*. Thus, the break in reproduction (and perhaps the occurrence of oosorption as well) appeared to enhance the females’ reproductive capability relative to females that had been reproducing continuously. Ohgushi (1996) reported that the survivorship of herbivorous lady beetles was enhanced by oosorption (accompanied by a break in reproduction) in response to flooding of their habitat. Our data also support the general hypothesis that oosorption can be a key element in the trade-offs between current and future reproduction (e.g., Bell and Bohm 1975).

Molecular tools have been used to study insect oosorption (e.g., Kurihara 1975; Maeta et al. 1981; Kotaki 2003, Asplen and Byrne 2006). However, the mechanisms of

oosorption and the question of how females can improve their survivorship or future reproduction by oosorption is not yet well understood. Relative enhancement of reproduction following temporary cessation of egg-laying in the absence of prey, is suggested by the results presented here, but further studies will be needed to understand the study of ovarian dynamics and the mechanisms of oosorption.

In summary, the results of the experiments presented here support the hypothesis that females of *C. septempunctata* may respond more quickly in their reproductive effort, than females of *C. transversoguttata*, to removal of prey in an experimental setting. The data from dissection also indicate that oosorption occurs in both species, although clear differences were not apparent between the two species (e.g., in the number of oocytes that may have been resorbed). Females of the invasive *C. septempunctata* recovered more fully in their rates of reproduction after six days of starvation than did females of the native *C. transversoguttata*. These differences between the two species could be related to their differential success in reproducing in environments in which prey availability fluctuates greatly over space and time, such as in alfalfa fields where *C. septempunctata* has become the dominant lady beetle present.

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Table 5-1 Results of two-way repeated measures of ANOVA of egg volume and egg viability in 2006 and 2007 (year effect) for both *Coccinella septempunctata* and *C. transversogutatta* (species effect) on Days 15–19 of experiments (day effect).

<i>Egg volume</i>				<i>Egg viability</i>			
	<i>DF</i>	<i>F</i>	<i>P</i>		<i>DF</i>	<i>F</i>	<i>P</i>
Year	1, 13.1	0.86	0.3695	Year	1,147	8.35	0.0044
Species	1, 13.1	34.33	<0.0001	Species	1,147	0.53	0.4671
Year x Species	1, 13.1	0.06	0.8099	Year x Species	1,147	1.62	0.2049
Day (Day 15–Day 19)	4, 30.4	14.55	<0.0001	Day (Day 15–Day 18)	3,160	24.42	<0.0001
Year x Day	4, 30.4	6.28	0.0008	Year x Day	3,160	1.29	0.2790
Species x Day	4, 30.4	2.91	0.0379	Species x Day	3,160	3.55	0.0158
Year x Species x Day	4, 30.4	1.62	0.1946	Year x Species x Day	3,160	0.06	0.9827

Table 5-2 Results of three-way ANOVA on the number of mature oocytes and the volumes of the most developed follicles in 2006 and 2007 (year effect) for both *Coccinella septempunctata* and *C. transversogutatta* (species effect) on Days 16–21 of experiments (day effect).

<i># Mature oocytes</i>				<i>Sizes of follicles</i>			
	<i>DF</i>	<i>F</i>	<i>P</i>		<i>DF</i>	<i>F</i>	<i>P</i>
Year	1, 212	1.95	0.1643	Year	1, 210	0.84	0.3606
Species	1, 212	3.40	0.0664	Species	1, 210	1.28	0.2595
Year x Species	1, 212	0.17	0.6787	Year x Species	1, 210	0.08	0.7724
Day (Day 16–Day 21)	5, 212	29.10	<0.0001	Day (Day 16–Day 21)	5, 210	48.67	<0.0001
Year x Day	5, 212	1.34	0.2485	Year x Day	5, 210	3.38	0.0058
Species x Day	5, 212	2.09	0.0678	Species x Day	5, 210	1.06	0.3832
Year x Species x Day	5, 212	1.93	0.0906	Year x Species x Day	5, 210	3.95	0.0019

Table 5-3 Results of three-way ANOVA for the number of mature oocytes present on one day versus net production (eggs laid + mature oocytes present) on the next day, for *C. septempunctata* and *C. transversoguttata* (species effect) in 2006 and 2007 (year effect) on Day 16 versus 17, or Day 17 versus 18 (day effect).

	<i>a) Day 16 vs. Day 17</i>			<i>b) Day 17 vs. Day 18</i>		
	<i>DF</i>	<i>F</i>	<i>P</i>	<i>DF</i>	<i>F</i>	<i>P</i>
Species	1, 75	3.53	0.0642	1, 70	4.94	0.0295
Year	1, 75	1.86	0.1764	1, 70	1.94	0.1677
Species × Year	1, 75	0.26	0.6084	1, 70	2.11	0.1504
Days	1, 75	13.37	0.0005	1, 70	8.19	0.0055
Species × Year	1, 75	1.98	0.1633	1, 70	0.82	0.3687
Year × Days	1, 75	0.04	0.8333	1, 70	0.39	0.5365
Species × Year × Days	1, 75	3.74	0.0568	1, 70	3.53	0.0644

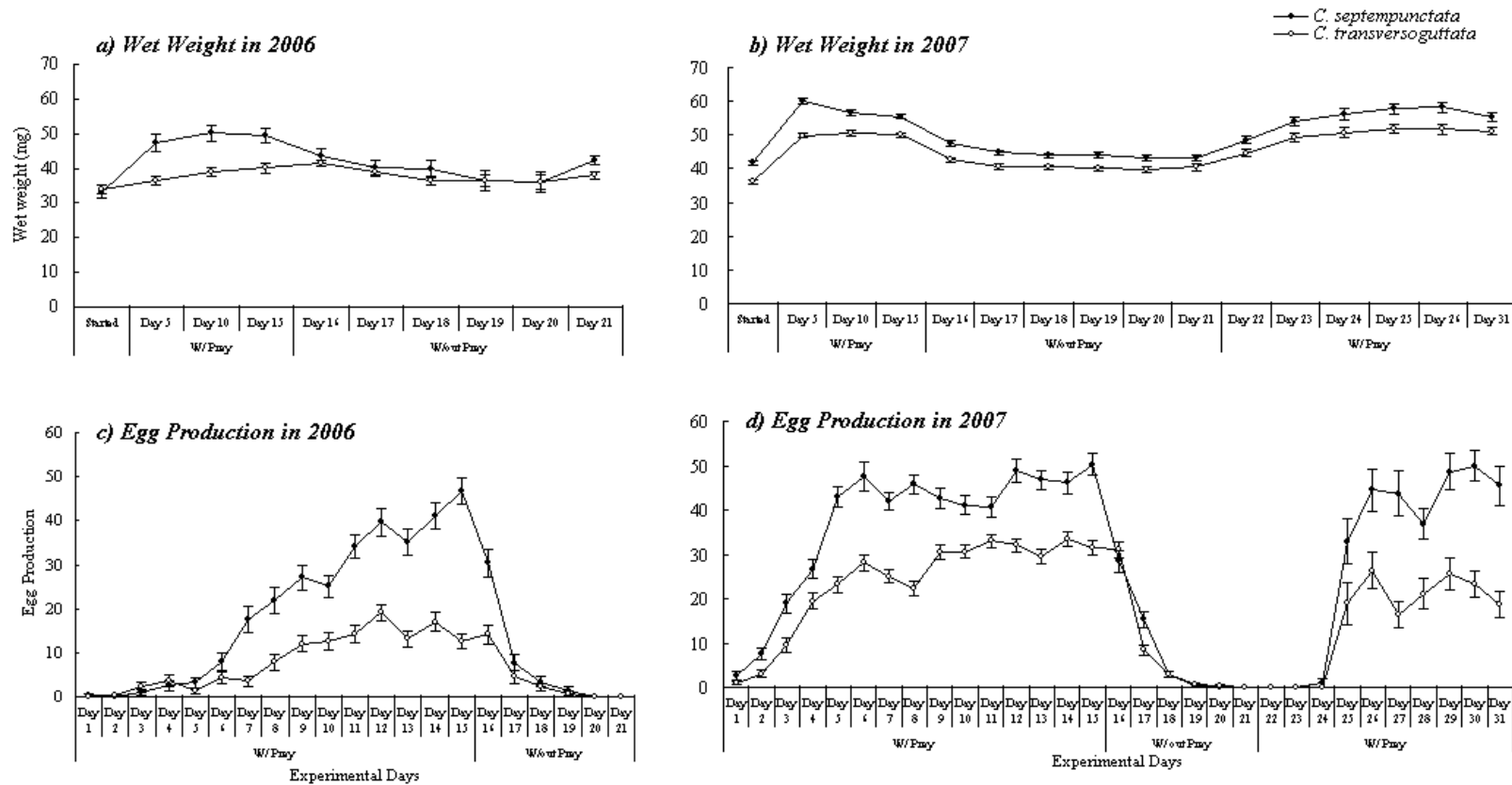


Fig. 5-1 Wet weight (\pm SE) of *Coccinella septempunctata* and *C. transversoguttata* in (a) 2006 and in (b) 2007, and egg production (\pm SE) of *C. septempunctata* and *C. transversoguttata* in (c) 2006 and in (d) 2007 during the experimental period.

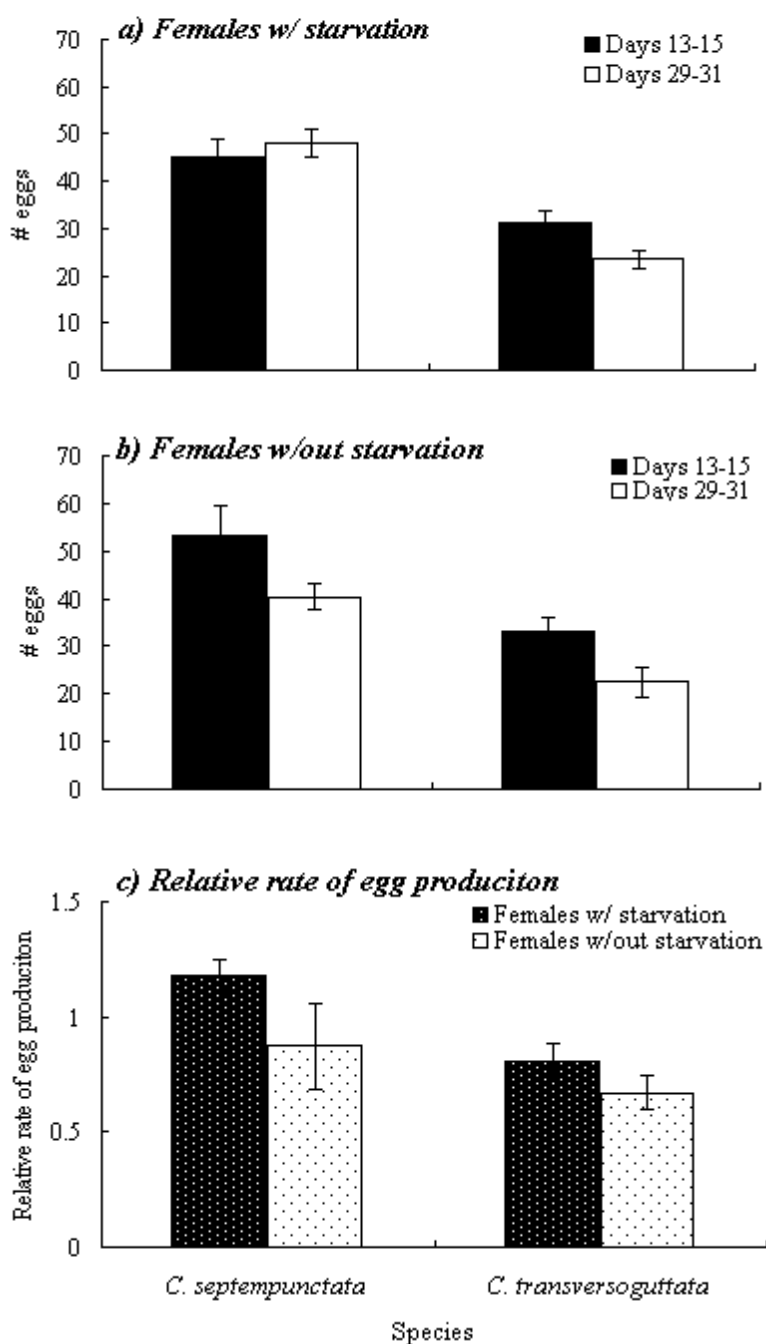


Fig. 5-2 Mean egg production per day (\pm SE) during Days 13–15 (maximum reproduction), and during Days 29–31 by (a) females that experienced starvation (i.e., removal of aphid prey) for 6 days (Days 16–21), (b) females that were provided with excess aphids throughout the entire 31-day experimental period, and (c) egg production rate during Days 29–31 compared with egg production during Days 13–15.

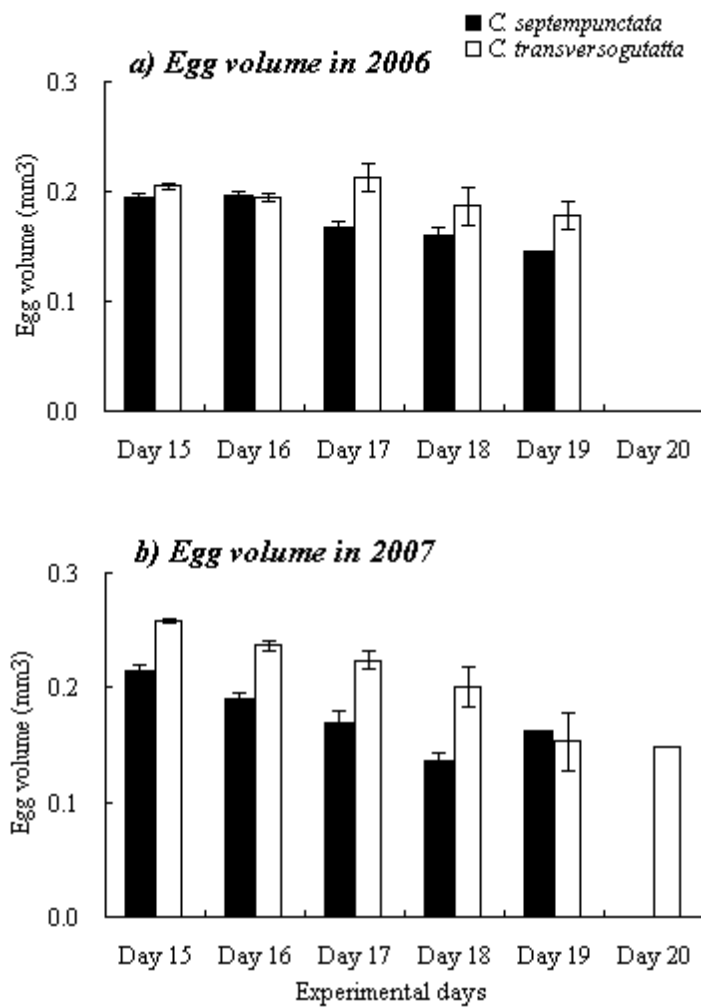


Fig. 5-3 Mean egg volume (mm³) [\pm SE] of *Coccinella septempunctata* and *C. transversoguttata* during the experimental period in (a) 2006 and in (b) 2007.

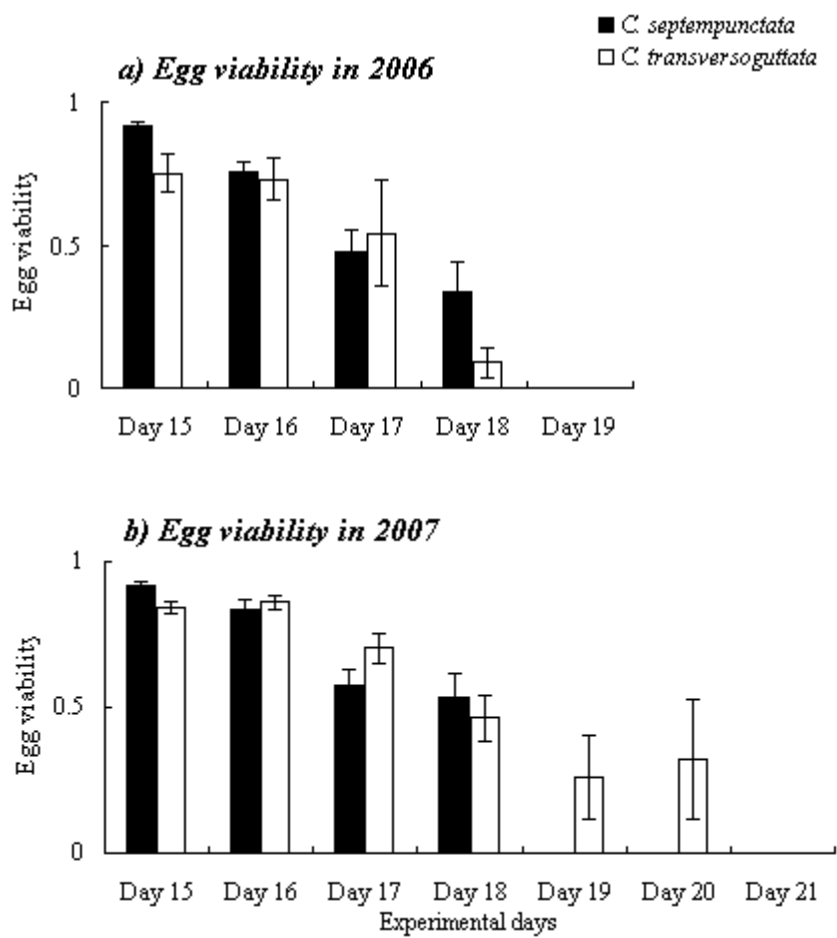


Fig. 5-4 Mean egg viability [±SE] of *Coccinella septempunctata* and *C. transversoguttata* in (a) 2006 and (b) 2007.

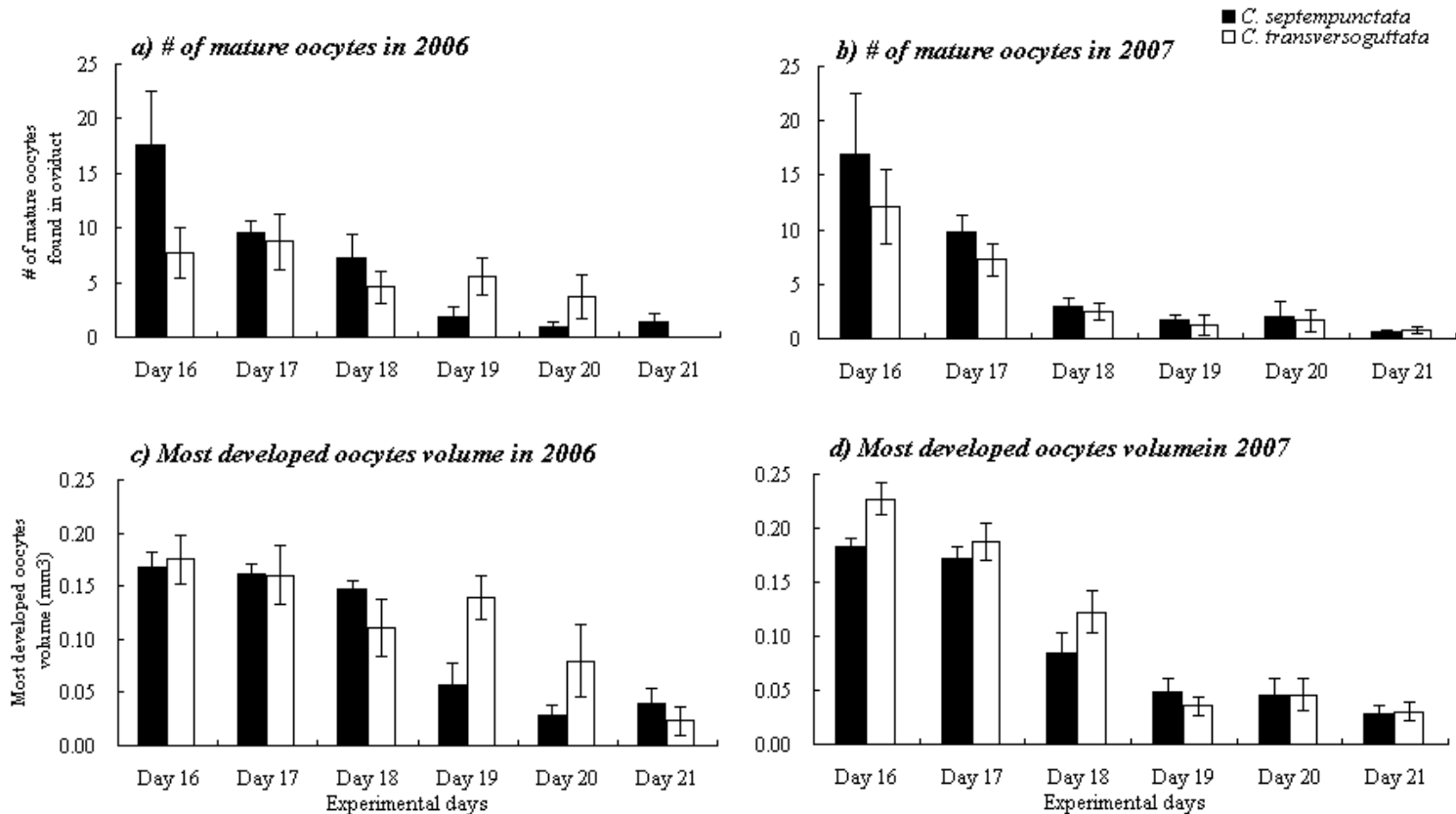


Fig. 5-5 Mean number of mature oocytes (\pm SE) of *Coccinella septempunctata* and *C. transversoguttata* found in the lateral oviducts (left and right combined) in (a) 2006, and (b) 2007; and the mean volume (\pm SE, in mm^3) of the six most developed follicles in ovarioles of *C. septempunctata* and *C. transversoguttata* in (c) 2006, and (d) 2007, on individual days without aphids during the experimental period.

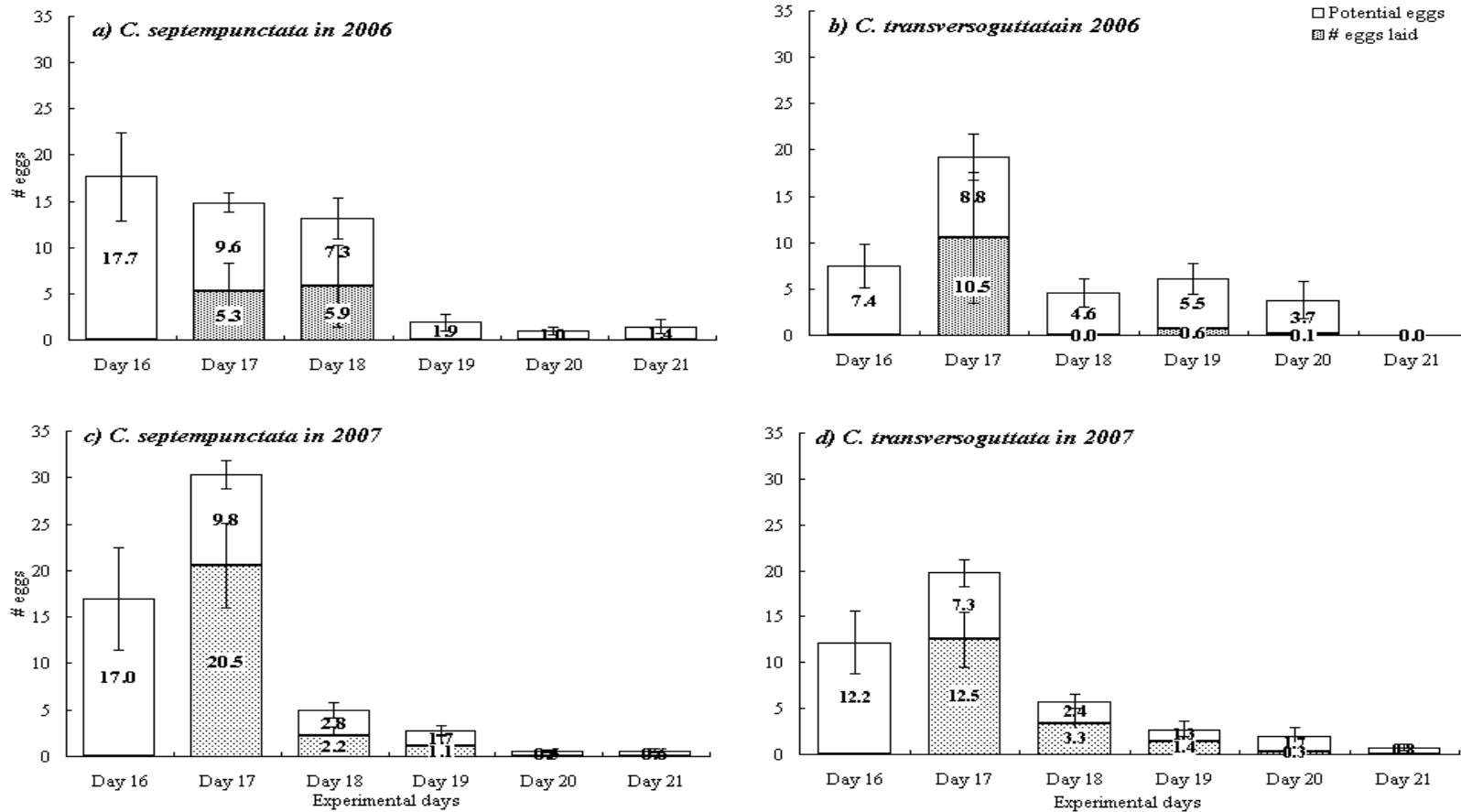


Fig. 5-6 Comparisons of the number of mature oocytes (\pm SE) in the oviducts (as determined at the end of each day), and the number of eggs (\pm SE) laid during each day of the starvation period (in which aphids were removed) for in (a) *Coccinella septempunctata* and (b) *C. transversoguttata* in 2006, and (c) *C. septempunctata* and (d) *C. transversoguttata* 2007. For example, 17.7 mature oocytes on average were present in the oviducts of *C. septempunctata* females at the end of Day 16 in 2006, and subsequently 5.3 eggs on average were laid over the next 24 hours (i.e., during Day 17). Note that the number of eggs laid on Day 16 (before the first sets of females were dissected) is omitted for these comparisons.

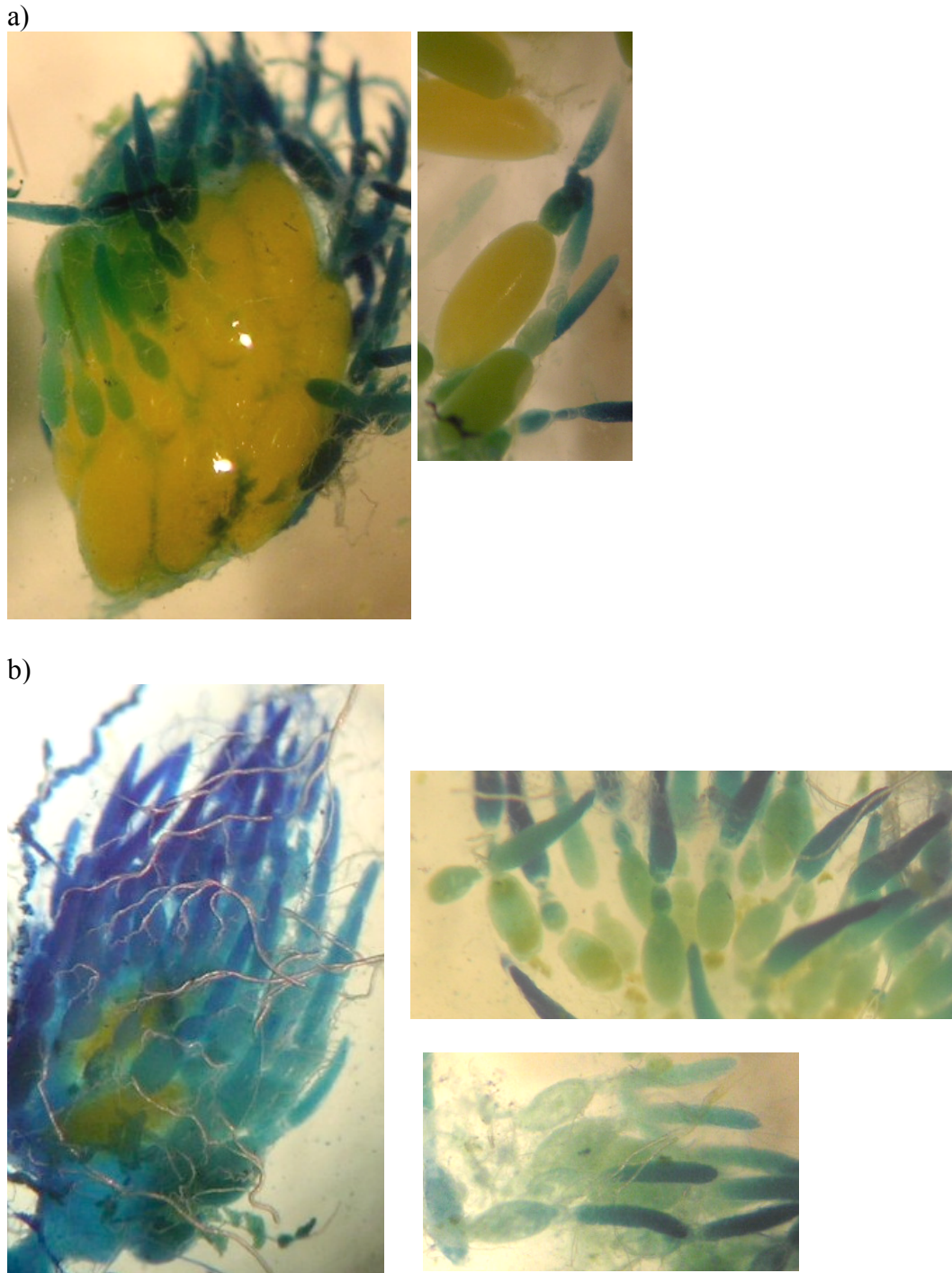


Fig. 5-7 (a) Fully developed ovary and ovariole of *Coccinella transversoguttata*, (b) ovary and ovarioles of *C. transversoguttata* during the no-prey condition.

CHAPTER 6

SUMMARY

Invasive species have attracted much attention in recent years because of their disruptive effects on biodiversity worldwide. Classical biological control, used instead of or with chemical pesticides in pest management, may pose risk through adverse non-target effects of exotic natural enemy introductions on native species and biodiversity in local communities (e.g., Howarth 1991; Simberloff and Stiling 1996; Thomas and Willis 1998; Louda et al. 2003). However, it is often not well understood how local communities and ecosystems are disturbed by invasive species, and which mechanisms contribute to successful establishment of invasive species.

Predatory lady beetles (or ladybirds; Coleoptera: Coccinellidae) have been introduced to North America for agricultural pest management in recent decades, raising concerns of adverse impact on native species, especially native lady beetles (e.g., Evans 1991; Louda et al. 2003). A prominent species among these introduced lady beetles is *Coccinella septempunctata* (Angalet et al. 1979). There are several reports that the densities of native predatory lady beetles, especially *C. transversoguttata*, have declined as these species have been displaced by the invasive *C. septempunctata* in North America (e.g., Elliott et al. 1996; Turnock et al. 2003; Alyokhin and Sewell 2004). In northern Utah, *C. septempunctata* first appeared in 1991, and now it is predominant among predatory lady beetles in alfalfa (Evans 2000, 2004). How invasive species reproduce as well as survive in a new environment is significant for their establishment. Therefore, I conducted studies to compare reproductive habits and tactics of the invasive *C. septempunctata* with those of *C. transversoguttata*, a closely related native species that it

has been displaced. I addressed two general questions for my dissertation: 1) how are the reproductive tactics of lady beetles adapted to exploiting their prey? and 2) do the two species differ in their reproductive tactics in ways that may contribute to species displacement?

To seek answers for these two general questions, I pursued studies that are reported in this dissertation. These studies were organized into four main chapters that present comparisons of reproductive tactics between the invasive *C. septempunctata* and the native *C. transversoguttata* (and other three native *Hippodamia* species for Chapter 2) for the following topics:

- 1) Reproductive tactics under excess food condition,
- 2) Seasonal reproductive patterns under natural condition,
- 3) Reproductive tactics and plasticity under limited prey availabilities,
- 4) Ovarian dynamics and observation of oosorption under no-prey condition.

Reproductive tactics under excess food condition

Overwintered adults of the invasive lady beetle, *C. septempunctata* and the native lady beetle, *C. transversoguttata*, as well as three other native lady beetles (*Hippodamia convergens*, *H. quinquesignata*, and *H. sinuata*), were compared for reproductive rates when provided with excess numbers of aphids. The relationships between body size, fecundity and invasion success were examined.

Among species, the number and total volume of eggs (number x mean egg volume) produced per day increased with increasing female size and were greatest for *C. septempunctata* as the largest species. Rates of reproduction also increased with

increasing female body size within a species. Although there was considerable overlap in body size, females of *C. septempunctata*, on average, were larger than females of the largest native species, *C. transversoguttata*. Females of two *Coccinella* species that were the same size laid similar total volume of eggs per day, but females of the invasive species had more ovarioles and laid larger numbers per day of individually smaller eggs. Thus, it appears that the invasive lady beetle, *C. septempunctata*, gains a reproductive advantage over native, North American lady beetles from its larger body size when feeding on abundant prey in a habitat in which it predominates. The invasive species may gain additional advantage in this habitat over the similarly sized, congeneric, native species *C. transversoguttata*, through its allocation of resources to larger numbers of relatively smaller eggs.

Seasonal reproductive patterns under natural condition

Over a 3-year period, field censuses were conducted to understand the population dynamics of lady beetles and their aphid prey, and the seasonal reproductive patterns. The suitability of alfalfa fields as habitat for lady beetles was examined by comparing reproduction of overwintering females of *C. septempunctata* and the native, similarly sized, and congeneric species *C. transversoguttata*. Populations of aphids in alfalfa fields increased from late April through the spring and early summer, and then decreased after the first cutting of hay and remained low thereafter. Data for egg production revealed a significant difference in the timing and amount of reproduction between *C. septempunctata* and *C. transversoguttata*. Females of *C. septempunctata* reproduced more readily and laid more eggs than *C. transversoguttata* females even at low prey

density. Early reproductive activity and high fecundity in the invasive *C. septempunctata* was observed consistently among fields and years. The results, when compared to lady beetle performance in the laboratory under ideal conditions, also indicated that in alfalfa fields, *C. septempunctata* females were more successful in approaching their maximum body weights and reproductive output than were females of native *C. transversoguttata*. These results suggest that alfalfa fields may serve as source habitats for the invasive *C. septempunctata* and may be important breeding sites for this species to produce large numbers of adults that disperse widely among many habitats.

Reproductive tactics and plasticity under limited prey availabilities

Laboratory experiments were conducted to compare the reproductive responses of females of the two species of lady beetles, *C. septempunctata* and *C. transversoguttata*, to varying availability of prey. Overall, the results showed that females of *C. septempunctata* and *C. transversoguttata* respond similarly in the total volume of eggs that they lay when they consume pea aphids in varying amounts. In particular, the experiments provided no evidence that females of *C. septempunctata* convert pea aphids into eggs at a relatively higher rate than females of *C. transversoguttata* do when prey availability is limited. On the other hand, when lady beetles were placed immediately upon experimental diets after being collected from the field, females of *C. septempunctata* produced more (but individually smaller) eggs than did females of the similarly sized *C. transversoguttata*. Females of *C. septempunctata* also had greater ability than *C. transversoguttata* females to maintain their body weights even as they were producing eggs at low rates when aphids were provided in limited numbers. This

suggests that low aphid availability is less stressful for reproducing females of *C. septempunctata*, perhaps because females of *C. septempunctata* have more ability physiologically to assimilate pea aphid nutrients (many of which are used for self-maintenance) at low aphid availability, compared with reproducing females of *C. transversoguttata*. Furthermore, overwintered females of *C. septempunctata* may arrive in alfalfa fields with greater nutrient reserves, thereby also enabling them to reproduce more quickly thereafter than females of *C. transversoguttata*.

Ovarian dynamics and observation of oosorption under no-prey condition

Ovarian and egg-laying dynamics of the two species of lady beetle, *C. septempunctata* and *C. transversoguttata*, were examined in two laboratory experiments in which aphid prey first were provided in excess for an extended period, and then were removed. *C. septempunctata* responded more quickly by reducing their reproductive effort than did females of *C. transversoguttata*, to removal of prey in an experimental setting. Data from dissection showed the potential occurrence of oosorption in both species; however, no clear differences between the two species were observed. In *C. septempunctata*, about 5 potential eggs (i.e., mature oocytes) resorbed in both 2006 and 2007, and in *C. transversoguttata*, about 4 and 1.8 potential eggs resorbed in 2006 and 2007, respectively, 2 to 3 days after prey removal. In the second experiment, aphids were provided again to experimental females after 6 days during which the prey were withheld. Females of both species started to reproduce 2 to 3 days after they again were provided with aphids in excess. Females of *C. septempunctata* laid almost the same number of eggs as they had before they were starved; however, females of *C. transversoguttata*

produced fewer eggs than they had before. Such immediate adjustment of reproductive effort when prey were removed, and high recovery of reproductive rate when prey again became available, as observed in *C. septempunctata* compared with *C. transversoguttata*, may contribute to the relative success of *C. septempunctata* in reproducing in alfalfa fields.

In conclusion, my study may support the hypothesis that invasive *C. septempunctata* has high reproductive rate. Invasive *C. septempunctata* have reproductive advantages under excess food availability because they have larger body size and more ovarioles which allow them to lay more, smaller eggs than *C. transversoguttata*. Such trade-offs between egg number and size were also important reproductive tactics in invasive *C. septempunctata* under limited prey availabilities. These results may support why invasive *C. septempunctata* reproduced more successfully under natural condition. In addition, females of *C. septempunctata* maintained their body weights even as they were producing eggs at low rates when aphids were provided in limited numbers. Females of *C. septempunctata* responded more quickly by reducing their reproductive effort than females of *C. transversoguttata* in response to fluctuation in prey availability. Both results may suggest that invasive *C. septempunctata* have more efficient physiological capacity to tolerate to environmental variations. My study may provide insight into why the introduced lady beetle has become much more abundant than its native congener in this habitat.

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septempunctata. Can Entomol 135:391–404

CURRICULUM VITAE

YUKIE KAJITA

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PERSONAL INFORMATION

Citizenship: JAPAN

Contact Information in JAPAN: 3-171-7 Kurume, Koriyama, Fukushima 963-8846

EDUCATION

Ph.D. Biology and Ecology, Utah State University, Logan, August in 2008

Advisor: Dr. Edward (Ted) W. Evans

Dissertation: Reproductive tactics of aphidophagous lady beetles: Comparison of a native species and an invasive species that it displacing it.

M.S. Agriculture and Entomology, Yamagata University, Tsuruoka, March in 2002

Advisor: Dr. Hironori Yasuda

Thesis: Effects of two predatory ladybirds (Coleoptera: Coccinellidae) on the guild structure of aphidophagous arthropods.

B.S. Agriculture and Entomology, Yamagata University, Tsuruoka, March in 2000

Advisor: Dr. Hironori Yasuda

Thesis: Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species.

RESEARCH EXPERIENCE

**Visiting Scientist: US-Japan Cooperative Science Program (NSF INT-0089374)
Utah State University, Department of Biology, Logan, Utah
August–September in 2001**

Collaborated with Dr. E.W. Evans (Utah State University, U.S.A.) and Dr. Hironori Yasuda (Yamagata University, Japan) on the research of predatory lady beetles which had been introduced to the U.S. as biological control agents 30 years ago and the effects of which are currently of great interest.

Research Assistant

Utah State University, Department of Biology, Logan, Utah

May–August in 2004–2008

Worked with Dr. E.W. Evans on the research of biological control of invasive Canadian thistles in Utah, and the biological control of cereal leaf beetles.

TEACHING EXPERIENCE

Teaching Assistant

Utah State University, Department of Biology, Logan, Utah

September–April in 2003–2008

Worked in the general Biology Lab (Biol 1210/1220, Biol 1610/1620). Prep work for lab setting, writing lecture, and making exams.

Worked in the Field Ecology class (Biol 3220). Assistant for data collecting in fields and data analyses.

College Preparatory Tutor
Koriyama, Fukushima, Japan
May in 2002–July in 2003

Tutor high school and middle school students in preparing for their respective entrance exams as well as in their regular schoolwork in Math, Science, Japanese, English, and History.

Teaching Assistant
Yamagata University, Tsuruoka, Yamagata, Japan
April in 2000–March in 2002

Assistes Professor Hironori Yasuda in conducting two laboratory classes per semester (Insect Ecology and Applied Entomology), including lab preparation, equipment training, and demonstrations.

Tutor
Tsuruoka, Yamagata, Japan
July in 1997–March in 2002

Tutor junior high school students in preparing for their respective entrance exams as well as in their regular schoolwork in Math, Science, Japanese, English and History.

ACADEMIC AWARDS

Japan Scholarship Foundation (Ikuei-kai) in 1996–2002

Received monthly stipends (\$440) for four years of undergraduate and (\$1,000) for two years of graduate education. Selected based on application essay and academic excellence.

Ecology Center Graduate Research Support Awards, Utah State University,
\$2,500 in 2005–2006, \$2,200 in 2006–2007

The research awards for MS and PhD graduate students pursuing ecology degrees to support thesis / dissertation research. The grants are competitive and selection is based on scientific merit, quality of the proposals, and relevance to the field of ecology.

James A. and Patty MacMahon Scholarship 2006, Utah State University (\$1,000)
in 2006–2007

The scholarship is for the graduate students in Biology who are seeking Ecology degree in Utah State University. Selected based on the students statements and academic excellence.

IOBC Fellowship (International Organization of Biological Control)
Student Travel Grants (800 euros) in 2007

The fellowship for the meeting of the Ecology of Aphidophaga 10, Athens, Greece, September 6–10 in 2007. Selected based on the research abstract.

P U B L I C A T I O N S

Kajita Y, Takano F, Yasuda H (2000)

Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of exotic species in relation to prey abundance. *Appl Entomol Zool* 35:473–479

Kajita Y, Takano F, Yasuda H, Evans EW (2006)

Effects of native ladybirds on oviposition of the exotic species, *Adalia bipunctata* (Coleoptera: Coccinellidae) in Japan. *Appl Entomol Zool* 41:57–61

Kajita Y, Takano F, Yasuda H, Evans EW (2006)

Interactions between introduced and native predatory ladybirds (Coleoptera: Coccinellidae): factors influencing the success of species introductions. *Ecol Entomol* 31:58–67

Kajita Y, Yasuda H, Evans EW (in prep)

Dynamic food web structure driven by top predator, *Harmonia axyridis* (Coleoptera, Coccinellidae).

Kajita Y, Evans EW (in prep)

Reproductive comparison between an invasive and native species: relationship of body size, fecundity and invasion success.

Kajita Y, Evans EW (in prep)

Reproductive comparison between an invasive and a native lady beetle in alfalfa fields in response to prey population dynamics.

Kajita Y, Evans EW (in prep)

Comparative reproduction of two lady beetles at low versus high prey density.

Kajita Y, Evans EW (in prep)

Ovarian Dynamics and observation of oosorption in two predatory lady beetles (Coleoptera: Coccinellidae).

Agarwala BK, Yasuda H, **Kajita Y** (2003)

Effect of conspecific and heterospecific faeces on foraging and oviposition of two predatory ladybirds: role of faecal cues in predator avoidance. *J Chemical Ecol* 29:357–376

Yasuda H, Evans EW, **Kajita Y**, Urakawa K, Takizawa T (2004)

Asymmetric larval interactions between introduced and indigenous ladybirds in North America. *Oecologia* 141:722–731

Kindlmann P, Yasuda H, **Kajita Y**, Dixon AFG (2005)

Field test of the effectiveness of ladybirds in controlling aphids. In: Hoddle MS (ed) *International symposium on biological control of arthropods*, September 12-16, 2005, Davos, Switzerland, USDA Forest Service, pp 441–447

Kindlmann P, Yasuda H, Sato S, **Kajita Y**, Dixon AFG (in prep)

Field test of the egg window hypothesis using a ladybird-aphid system.

P R E S E N T A T I O N S

“Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species” (Bachelor Thesis)

- Oral presentation at the 7th International Symposium of Ecology of Aphidophaga, August 31-September 4 in 1999, Quebec, Canada.
- Oral presentation at the 44th Annual Meeting of the Japanese Society of Applied Entomology and Zoology, March 29–31 in 2000, Ibaraki, Japan.
- Oral Presentation at the 88th Pacific Branch of the Entomological Society of America, June 20–23 in 2004, Bozeman, Montana, U.S.A.

“Effects of two predatory ladybirds (Coleoptera: Coccinellidae) on the aphidophagous arthropods” (Master Thesis)

- Oral presentation at the 45th Annual Meeting of the Japanese Society of Applied Entomology and Zoology, March 31–April 2 in 2001, Shimane, Japan.
- Oral presentation at the 49th Annual Meeting of the Ecological Society of Japan, March 25–30 in 2002, Sendai, Japan.
- Oral presentation at the 46th Annual Meeting of the Japanese Society of Applied Entomology and Zoology, March 28–30 in 2002, Tokyo, Japan.
- Poster presented at 8th International Symposium of Ecology of Aphidophaga, September 1–6 in 2002, Ponta Delgada Azores, Portugal.
- Oral Presentation at the 52nd Annual meeting of the Entomological Society of America, September 14–17 in 2004, Salt Lake City, UT, U.S.A.
- Oral Presentation at the 1st International Symposium of Biological Control of Aphids and Coccids, September 25–29 in 2005, Tsuruoka, Yamagata, Japan.

“Reproductive tactics of aphidophagous ladybirds of the genus *Coccinella*: Comparison of a native species and a competing invasive species” (Ph.D. Dissertaion)

- Oral Presentation at the 54th Annual meeting of the Entomological Society of America, December 10–13 in 2006, Indianapolis, Indiana, U.S.A. (Travel expenses were supported by James A. and Patty MacMahon Scholarship in 2006, Utah State University).
- Oral Presentation at the Ecology of Aphidophaga 10, September 6–10 in 2007, Athens, Greece. Travel expenses were supported by IOBC fellowship and the Ecology Center, Utah State University.
- Oral Presentation at the 55th Annual meeting of the Entomological Society of America, December 9–12 in 2007, San Diego, California, U.S.A.

RESEARCH INTERESTS

- Biological control
- Predator-prey interaction
- Studies in agro-ecosystems
- Invasion ecology and community ecology
- Insect physiology, ecology and life-history traits

PROFESSIONAL MEMBERSHIPS

- Entomological Society of America, since 2004
- Ecological Society of Japan, 2001–2004
- Japanese Society of Applied Entomology and Zoology, 1999–2004

REFERENCES

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